

**CIRCUIT AND PHARMACOLOGICAL APPROACHES FOR
COUNTERACTING STRESS EFFECTS ON THE DOPAMINE SYSTEM:
IMPLICATIONS FOR MAJOR DEPRESSION**

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Major Depressive Disorder (MDD) is a leading cause of disability worldwide. Evidence from clinical and preclinical models suggests that an important component to MDD is dysfunction in the mesolimbic dopamine reward system. This thesis focused on better understanding the brain circuits potentially involved in driving this dopamine system dysfunction, as well as mechanisms of an antidepressant therapy that directly acts on the dopamine system. We used the chronic mild stress (CMS) rodent model of depression, which features a prominent hypodopaminergic phenotype, to answer two specific questions: 1) How do brain regions with identified involvement in MDD based on clinical literature regulate the dopamine system in normal and CMS-exposed rats; and 2) What effect does the atypical antipsychotic agent quetiapine have on normal and CMS-exposed rats with acute and repeated administration? In each case, we performed single-unit recordings of identified ventral tegmental area (VTA) dopamine neurons to assess the dopamine system's functional status. For question 1, we found that activation of the Infralimbic Prefrontal Cortex (ILPFC; rodent homologue of human Brodmann Area 25) and Lateral Habenula (LHb) in normal rodents each inhibits a distinct subset of dopamine neurons based on their location in the VTA, that those inhibited by the ILPFC have greater overlap with those inhibited by CMS than do those inhibited by the LHb, and that ILPFC but not LHb inactivation following CMS restores dopamine system function to normal levels. For question 2, we found that quetiapine has distinct effects on the dopamine system based on duration of administration and history of exposure to CMS, with only repeated quetiapine administration restoring dopamine system function in CMS-exposed rats. Together, these results offer insight to clinically relevant questions regarding interactions among brain regions mediating hedonic and affective processes, as well as highlight potential dopaminergic mechanisms of novel antidepressant therapies.

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ABBREVIATIONS

%SIB: Percent of Spikes in Burst

APD: Antipsychotic Drug

BA25: Brodmann Area 25

BLA: Basolateral Amygdala

CMS: Chronic Mild Stress

CPT: Cells Per Track

D2R: Dopamine receptor type 2

FST: Forced Swim Test

ILPFC: Infralimbic Prefrontal Cortex

LHb: Lateral Habenula

MDD: Major Depressive Disorder

RDoC: Research Domain Criteria

RMTg: Rostromedial Tegmental Nucleus

SPT: Sucrose Preference Test

VP: Ventral Pallidum

vSub: Ventral Subiculum of the Hippocampus

VTA: Ventral Tegmental Area

PREFACE

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“The first thing that goes is happiness. You cannot gain pleasure from anything...But soon other emotions follow happiness into oblivion: sadness as you had known it, the sadness that seemed to have led you here; your sense of humor...Your mind is leached until you seem dim-witted even to yourself...Eventually, you are simply absent from yourself.”

—Andrew Solomon,

The Noonday Demon: An Atlas of Depression

1.0 GENERAL INTRODUCTION

1.1 OVERVIEW OF DEPRESSION

1.1.1 Burden of Depression

Major depressive disorder (MDD) is a constellation of symptoms including not only sadness and/or anhedonia, but also changes in homeostatic function including eating, sleeping, and cognitive processes (American Psychiatric Association, 2013). The disorder is very common, affecting an estimated 17% of the US population over the course of their lifetime (Kessler, Berglund, et al., 2005) and 7% of the population at any one given time (Kessler, Chiu, Demler, Merikangas, & Walters, 2005). Thus, as one might expect, the human impact of depression is significant. It is predicted to be the number one source of disability adjusted life years worldwide by the year 2030 (World Health Organization, 2008). This is due to a multitude of factors, including a relative lack of effective treatments and difficulty appropriately assigning a given treatment to a particular patient.

1.1.2 Limitations of Current Treatments

1.1.2.1 Antidepressant Medications

Unfortunately, depression is extremely difficult to efficiently treat. Only half of patients respond to an initial treatment with an antidepressant agent (Trivedi, Rush, et al., 2006), and only one third of patients recover from their depression with this medication. Subsequent medication attempts result in similarly low rates of remission, regardless of the pharmacological mechanism of the second agent selected (Rush et al., 2006; Trivedi, Fava, et al., 2006). Thus a substantial proportion of the population experiences treatment resistant depression, defined as a failure to respond to at least 4 distinct medications (Nemeroff, 2007).

Time course is another major limitation of current antidepressant medications. Even when medications are effective, often it takes a patient weeks to months to see meaningful clinical improvement (Ressler & Nemeroff, 2000). For this reason, there has been significant excitement around the recently identified potential antidepressant effects of ketamine (Aan Het Rot, Zarate, Charney, & Mathew, 2012). When administered at subanesthetic doses, ketamine can lead to a rapid antidepressant effect, which starts typically within 2 hours and may persist as long as 2 weeks (Berman et al., 2000). While this prospect is exciting, a major fundamental problem remains in that ketamine's antidepressant effect was discovered serendipitously during its use in other investigational means (Krystal et al., 1994). Thus, there exists a major limitation in current antidepressant research (and in psychiatry more broadly), in that there is an over reliance on serendipity in the identification of new psychotropic compounds.

1.1.2.2 Device-Based Therapies

Many novel invasive and non-invasive brain stimulation devices for treating depression under development, including transcranial magnetic stimulation, vagus nerve stimulation, and deep brain stimulation (Moreines, McClintock, & Holtzheimer, 2011). Such approaches are appealing because they offer the ability to focally target abnormal neural circuits (Ressler & Mayberg, 2007). Moreover, electroconvulsive therapy, at present the most widely used clinical tool for brain stimulation in depression, offers very high levels of efficacy in a fairly rapid time course (Kho, van Vreeswijk, Simpson, & Zwinderman, 2003). However, its use is limited by neuropsychological side effects, thought to be due to the lack of focality in the stimulation delivered to the brain (Sackeim et al., 2007).

Deep brain stimulation emerged as a particularly exciting tool given the potential to focally target the treatment to highly localized brain regions and the high levels of success with using the approach in other neuropsychiatric diseases (Holtzheimer & Mayberg, 2011a). However, despite success in early studies (Holtzheimer et al., 2012), many of the most promising investigational devices have encountered significant difficulties in late phase clinical trials. Multiple reasons for these failures have been proposed (Widge, Deckersbach, Eskandar, & Dougherty, 2016), including a lack of consistently reliable means for identifying appropriate patients (Filkowski, Mayberg, & Holtzheimer, 2016; Riva-Posse, Holtzheimer, Garlow, & Mayberg, 2013), rush to complete clinical trials before reliable biomarkers to guide device implantation were identified (Riva-Posse et al., 2014), and clinical trial designs that did not accurately predict and account for the extent of placebo response in highly treatment-resistant patients .

Thus, despite significant efforts and excitement, there remain no commercially available device-based antidepressant approaches, aside from electroconvulsive therapy, that have

significantly greater antidepressant response likelihood or speed than traditional antidepressant medications.

1.1.3 New Approach to Research: RDoC

An examination of the status of medication development for depression, and psychiatric disorders more broadly, has identified a paucity in novel medication mechanisms as compared to other fields of medicine (Insel & Scolnick, 2006). Thus, despite an exponential increase in the number of medications now sold, many are just slight variations on existing compounds. In the case of depression, and many other psychiatric disorders, it has become clear that the single diagnosis is in effect an amalgamation of many different diseases with a final common end point, and these subtypes likely require different approaches to treatment (Holtzheimer & Mayberg, 2011b). To help redirect research into novel treatments for psychiatric disorders, the NIMH created the Research Domain Criteria (RDoC), with the purpose of identifying specific domains of pathology within and across existing diagnoses, such as apathy or psychosis (Insel et al., 2010). The ultimate goal would be to assign classes of medications to the treatment of specific functional symptoms, as opposed to the existing research model of identifying a single medication to treat all aspects of a particular mental illness (O'Donnell & Ehlers, 2015). Such has been a successful approach in other fields of medicine such as cardiology, where heart failure is treated with a combination of agents that counteract the neurohumoral changes associated with the disease including beta blockers, angiotensin converting enzyme-inhibitors/angiotensin receptor blockers, diuretics, aldosterone antagonists, and other novel targets, each which are selected based on a defined set of factors including ejection fraction, fluid volume status, and functional class of disability (Yancy et al., 2016).

1.1.3.1 Positive Valence systems of the RDoC

Within the RDoC construct, the Positive Valence Domain subcategory Approach Motivation is highly relevant to anhedonia, i.e. the lack of interest in previously pleasurable activities (NIMH RDoC Working Group, 2011). Moreover, the mesolimbic dopamine system is highly implicated in this process, suggesting that dopamine system dysfunction could be involved in the impairment in motivational and hedonic functions in MDD (Salamone & Correa, 2012). However, the relationship between the dopamine system and other brain regions with established roles in depression is currently poorly defined. We have identified that in other psychiatric disorders involving dopamine system dysfunction, the pathology is more a *dysregulation* of the dopamine system, rather than inherent pathology of the dopamine neurons themselves per se as is seen in neurodegenerative conditions such as Parkinson's disease (Grace, 2016). Thus, a better understanding is needed of how MDD circuit hubs influence dopamine system activity in normal and pathological states resembling MDD.

1.1.4 Dopamine and Behavior

Dopamine is a catecholamine tied to a wide variety of functional tasks ranging from movement, to motivation, to cognition depending on the pathway and site of its release (Bjorklund & Dunnett, 2007). Contrasting results of nigrostriatal dopamine neuron dysfunction are Parkinson's disease and Huntington's disease, wherein diminished and enhanced dopamine neuron activation causes abnormally decreased and increased movement, respectively (S. D. Iversen & Iversen, 2007). In schizophrenia, over-activation of mesolimbic dopamine neurons has been tied to paranoia and psychosis (Kapur, 2003). Furthermore, all known drugs of abuse enhance dopamine release (Bonci, Bernardi, Grillner, & Mercuri, 2003). So diverse are the functions mediated by dopamine

that entire textbooks have been dedicated to this neurotransmitter (L. L. Iversen, Iversen, Dunnett, & Bjorklund, 2009). Thus for the purpose of this thesis, discussion is focused to elements of the dopamine system related to motivational dysfunction in depression.

The role of dopamine in motivated behaviors is among the most fiercely debated topics in neuroscience. Among the earliest evidence for dopamine encoding motivationally relevant stimuli was the identification by Olds and Milner that stimulation of what was later identified to be the medial forebrain bundle resulted in highly reinforcing effects (Olds & Milner, 1954). Later, in a seminal body of work dissecting cortico-limbic-striatal circuitry relevant to motivated behaviors, Mogenson argued that mesolimbic dopamine projections were a critical element of the circuit for the “translation from motivation into action” (Mogenson, Jones, & Yim, 1980). Wise then advanced the anhedonia hypothesis, based on evidence that neuroleptics that block dopaminergic transmission will prevent the acquisition of reinforced motivated behaviors (Wise, 1982). This notion was further evaluated by Berridge and Robinson, who argued for an incentive salience function of dopamine wherein dopamine does not mediate the experience of pleasure per se (i.e., “liking”), but rather its pursuit (i.e., “wanting”) (Berridge & Robinson, 1998). A slight modification of this theory was later proposed by Salamone and Correa, who delineated the appetitive and activational aspects of wanting, and demonstrated that the latter are more sensitive to disruption of dopaminergic transmission (Salamone & Correa, 2002).

The above data were obtained primarily with interventions delivered *before* subjects interacted with the experimental stimuli (e.g. electrical stimulation or pharmacological blockade of dopamine pathways). However, a significant body of data also pertains to observing the effects of environmental stimuli on the firing responses of individual dopamine neurons as assessed with electrophysiology. This began after Schultz recorded from dopamine neurons in awake behaving

monkeys and observed that dopamine neurons tended to fire following the delivery of an unexpected reward (i.e. to signal a reward prediction error) (Schultz, Dayan, & Montague, 1997). This line of work has been continued in rodent models as well, particularly in computational and cognitive neuroscience circles, with significant interest in modeling dopamine neuron responses to given stimuli (Eshel et al., 2015).

In individuals with depression, deficits tend to be present in both the mobilization to pursue reward, and the enjoyment of its delivery (Treadway & Zald, 2011). Thus, details of the circuitry driving both of these deficits need to be more clearly elucidated.

1.1.5 Regulation of Dopamine Neuron Activity States

Over the past four decades, an enormous amount has been learned about the cellular mechanisms governing the different activation states of dopamine neurons, how these firing patterns are regulated by afferent circuitry, and where this circuitry may be disrupted in disorders involving abnormal dopamine neuron function (Grace, 2016).

1.1.5.1 Cellular Dynamics

The mechanisms governing firing properties of dopamine neurons were characterized in a series of papers by Grace and Bunney using intracellular recording of dopamine neurons identified as such via histofluorescence (Grace & Bunney, 1980). Dopamine neurons exhibit a slow depolarizing pacemaker potential (Grace & Bunney, 1983a), which originates at the soma (Grace & Bunney, 1983b), and couples between adjacent cells (Grace & Bunney, 1983c). These neurons can show two types of firing: an irregular firing pattern of single spikes dependent on membrane

voltage and variable degrees of after hyperpolarization (Grace & Bunney, 1984b), and burst firing driven by calcium entry (Grace & Bunney, 1984a).

However, not all neurons are spontaneously active, as some are in a non-firing state due to hyperpolarized membrane potentials (Grace & Bunney, 1983b). Only neurons that are exhibiting spontaneous spike activity are capable of transitioning to a burst mode, because burst firing is NMDA receptor dependent due to a calcium ion requirement (Chergui et al., 1993; Zweifel et al., 2009), and NMDA receptors are impermeable to calcium when membranes are in hyperpolarized states due to blockade by magnesium (Mayer, Westbrook, & Guthrie, 1984). Thus, the number of spontaneously firing dopamine neurons serves as the gain in regulating the magnitude of the dopamine system's phasic burst response to stimuli (Floresco, West, Ash, Moore, & Grace, 2003).

1.1.5.2 Afferent Circuitry

Further characterization of the different activity states of dopamine neurons has led to the discovery that different afferent circuits to the ventral tegmental area (VTA) regulate the number of spontaneously active dopamine neurons (i.e. dopamine population activity) and the amount of bursting occurring in these neurons (Lodge & Grace, 2006a). Burst firing is driven most potently by glutamatergic afferents from the pedunculopontine tegmentum (PPTg), gated by the laterodorsal tegmentum (Lodge & Grace, 2006b). Population activity is regulated by GABAergic afferents, among the most proximal and potent being the ventral pallidum (VP), which serves as a converging point to distinct circuits that have been shown to increase or decrease dopamine population activity (Grace, 2016) (Figure 1).

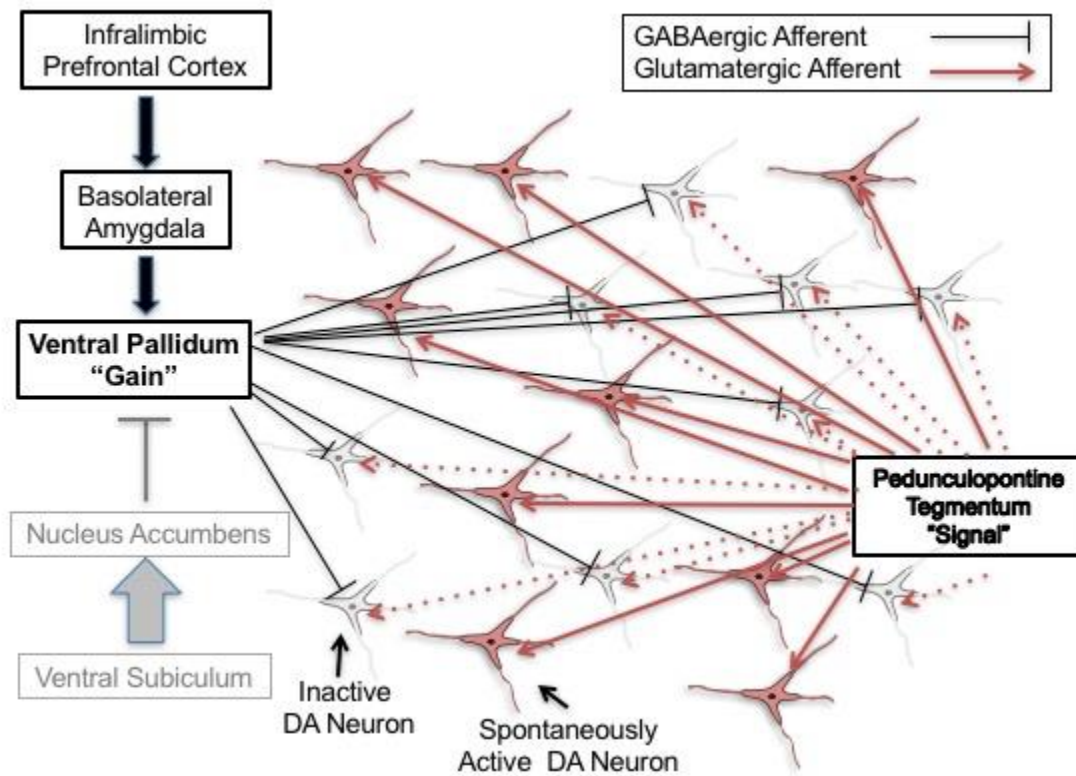


Figure 1. Circuits Regulating Dopamine Neuron Population Activity and Bursting

Polysynaptic circuits converging onto VP GABAergic afferents control the number of dopamine neurons that are spontaneously active, whereas the PPTg delivers glutamatergic signal that triggers burst firing.

At baseline states in awake or anesthetized preparations, only half of dopamine neurons are depolarized enough to exhibit spontaneous firing (Chiodo & Bunney, 1985), whereas the remaining neurons are hyperpolarized by afferent GABAergic circuitry. When the strength of afferent inhibitory drive is modulated (either increased or decreased), there is a corresponding inverse change in the number of spontaneously active dopamine neurons (Grace, Floresco, Goto, & Lodge, 2007). This can be quantified by performing 6-12 tracks of extracellular recording and calculating the number of spontaneously active dopamine neurons encountered per electrode track searched, yielding the metric of cells per track (Bunney & Grace, 1978).

Increased dopamine population activity occurs with activation of the ventral subiculum (vSub) of the hippocampus, which can be blocked when the glutamate antagonist kynurenic acid is infused into the NAc (Floresco, Todd, & Grace, 2001) or the GABA-A antagonist bicuculline is infused into the VP (Floresco et al., 2003). Together, these data suggest that the vSub drives an increase in dopamine population activity by enhancing NAc output, which is directed towards inhibition of the VP, thereby releasing more VTA dopamine neurons from VP inhibitory tone. Conversely, decreased dopamine population activity occurs with activation of the ILPFC, which can be blocked with kynurenic acid infusion in the BLA (Patton, Bizup, & Grace, 2013). BLA activation also reduces population activity, which is blocked by kynurenic acid infusion into the VP (Chang & Grace, 2014). This suggests that a circuit leading from the ILPFC through the BLA and VP inhibits dopamine population activity.

1.1.6 Dopamine Circuitry in Animal Models

The above circuits regulating dopamine neuron activity have been demonstrated to be highly relevant to animal models of psychiatric disorders involving increased or decreased dopamine system activity. The hyperdopaminergic state in schizophrenia has been studied using a neurodevelopmental model involving administration of the mitotoxin methazoxymethanol acetate (MAM) on day 17 of embryonic development (Moore, Jentsch, Ghajarnia, Geyer, & Grace, 2006). In this model, there is a marked increase in the number of spontaneously active dopamine neurons that is driven by pathological activation of the vSub (Lodge & Grace, 2007). In animal models employing chronic mild stress or amphetamine withdrawal, decreased dopamine neuron population activity has been identified, which is driven by heightened activation of the BLA (Belujon, Jakobowski, Dollish, & Grace, 2016; Chang & Grace, 2014). Collectively, these studies

in animal models of hyper and hypo dopaminergic states demonstrate the relevance of the circuits described above to disease states involving pathological changes in dopamine activity states.

1.2 EVIDENCE FOR DOPAMINE DYSFUNCTION IN DEPRESSION

The involvement of the dopamine system in MDD has been a focus of significant research effort in both clinical samples and animal models of depression. A selection of particularly important advances in both arenas are reviewed below.

1.2.1 Human Studies

Anhedonia is a core symptom of MDD (American Psychiatric Association, 2013) and is believed to be strongly tied to dysfunction of the dopamine system (Treadway & Zald, 2011). Measurements of dopamine metabolites in depressed patients showing reduced cortical dopamine turnover suggest tonic dopamine release is decreased in depression (Lambert, Johansson, Agren, & Friberg, 2000). Behavioral measurements of reward deficits in depression have found that patients show reduced capacity for acquiring rewarded behaviors (Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008). Using fMRI blood flow changes as a proxy measure for neuronal activation, studies of patients with MDD suggest dampened striatal responses to delivery of reward (Pizzagalli et al., 2009) or recollection of positive memories (Keedwell, Andrew, Williams, Brammer, & Phillips, 2005), which correlate with self-reported levels of anhedonia. Reduced responses in these regions also occur in trait anhedonia in subjects without MDD, suggesting that reductions in this pathway may mediate reward deficits more broadly (Gorwood, 2008).

Furthermore, healthy individuals demonstrate reduced striatal blood flow changes to reward when exposed to psychological stress while performing reward-based tasks (Kumar et al., 2014). Thus, in line with the RDoC, it has been suggested that examining the relationship between dopamine system dysfunction and anhedonia as a function of stress exposure across diagnoses could yield promising information (Pizzagalli, 2014).

1.2.2 Animal Studies

The first use of chronic stress to induce a depressive-like state in rodents was performed by Katz et al, in which rats were subjected to a 21-day period of persistently varied severe daily stressors including foot shock, forced swim in iced bath, prolonged (46-hour) food or water deprivation, tail pinch, shaker stress, and novel cage mates (Katz, Roth, & Carroll, 1981). This procedure was found to result in a number of clinically relevant behavioral deficits reminiscent of MDD including anxiety in an open field and reduced consumption of sucrose or saccharine solution, which were responsive to the tricyclic antidepressant imipramine (Katz, 1982) or electroconvulsive therapy (Katz, 1981). This procedure was later revised by Willner et al who observed that similar deficits could be obtained with far milder intensity stressors including changes in lighting, white noise, damp bedding, and shorter duration (22-hour) food and water deprivation (Willner, Muscat, & Papp, 1992), so long as the stressors were varied and unpredictable (Muscat & Willner, 1992).

From an early stage in the use of CMS, there was interest in the role of dopamine in driving anhedonic behavioral effects. Dopamine agonists were found to acutely reverse anhedonic like behavioral deficits (Willner et al., 1992), as were antidepressants targeting the dopamine system such as bupropion. More recently Tye et al demonstrated that optogenetic activation of VTA

dopamine neurons could reverse behavioral deficits induced by CMS (Tye et al., 2013). Thus, there is a clear role for dopamine system activation in ameliorating CMS-induced deficits in depressive-related behaviors.

1.2.2.1 Conflicting Evidence From Different Animal Models

The direction of change in dopamine activity became a topic of debate recently due to seemingly opposing results obtained using distinct animal models of depression (A. Grace & Moreines, 2014). The chronic social defeat (CSD) model of depression was originally developed in rats and adapted to mice (Miczek, Yap, & Covington, 2008). CSD involves subjecting an “intruder” mouse to exposure to a larger “resident” mouse in its home cage, which results in the intruder mouse being attacked by the larger resident mouse. This procedure is repeated daily for 10 days, after which the intruder mouse shows behavioral deficits relevant to depression including reduced sucrose preference and social interaction (Berton et al., 2006). In this model, a finding that appears to be critical to the behavioral phenotype is an increase in firing rate of VTA dopamine neurons (Cao et al., 2010). In contrast, rats exposed to the CMS or learned helplessness (LH) models of depression show reductions in the number of spontaneously active dopamine neurons, but not changes in dopamine neuron firing rates or bursting (Belujon & Grace, 2014; Chang & Grace, 2014).

The issue of differences in the apparent role of dopamine between the CSD and CMS depression peaked when two conflicting articles were published together in *Nature* showing that optogenetic activation of dopamine neurons enhanced the effects of CSD (Chaudhury et al., 2013), but reversed the effects of CMS (Tye et al., 2013), on depressive-related behaviors. To reconcile these differences, one must consider the distinct roles of dopamine in the induction vs. expression of depression. Acutely, severe stressors such as restraint stress *activate* the DA system (Valenti,

Lodge, & Grace, 2011), consistent with Chaudhury et al. (2013) who reported that increased DA neuron phasic activity *during* chronic social defeat stress (CSDS) was necessary for induction of the depressive phenotype that follows (Chaudhury et al., 2013). Thus, the initial increase in dopamine observed during and immediately after stress drives downstream plasticity, ultimately resulting in depression. In contrast, 24 hours after stress (e.g. acute restraint), there is a shift in the opposite direction, i.e. a *reduction* in dopamine neuron population activity (Chang & Grace, 2013). This is consistent with Tye et al. (2013), who observed that *after* CMS, phasic activation of dopamine neurons drove recovery from the depressive phenotype. Thus, in time, the dopamine up-regulation converts to down-regulation, and this hypoactivity causes depression-related behaviors (Belujon & Grace, 2014; Chang & Grace, 2014). Until recently, it was unclear whether CSDS caused this same overall down-regulation of the DA system. This question was indirectly addressed when the Chaudhury et al. group published in *Science* that activation of DA neurons long *after* CSDS has an *antidepressant* effect (Friedman et al., 2014), suggesting hypoactivation of the DA system may be present in the CSDS depression model as well. A second, related issue is that methodological differences in DA recording techniques between the CSDS studies and our findings preclude a direct comparison of the two lines of inquiry. CSDS has been demonstrated to increase *firing rate of individual DA neurons* (Krishnan et al., 2007), whereas its impact on the *population activity of the DA system* has not been assessed.

1.3 CLINICALLY IDENTIFIED NODES FOR INVESTIGATION

The work by Chang and Grace (2014) identified the BLA and VP as critical nodes in the downregulation of dopamine population activity after CMS. For the first portion of this thesis, we

examine additional brain regions with potential to be involved in the CMS-induced hypodopaminergic circuit (Figure 2) based on a synthesis of clinical and basic science literature discussed below.

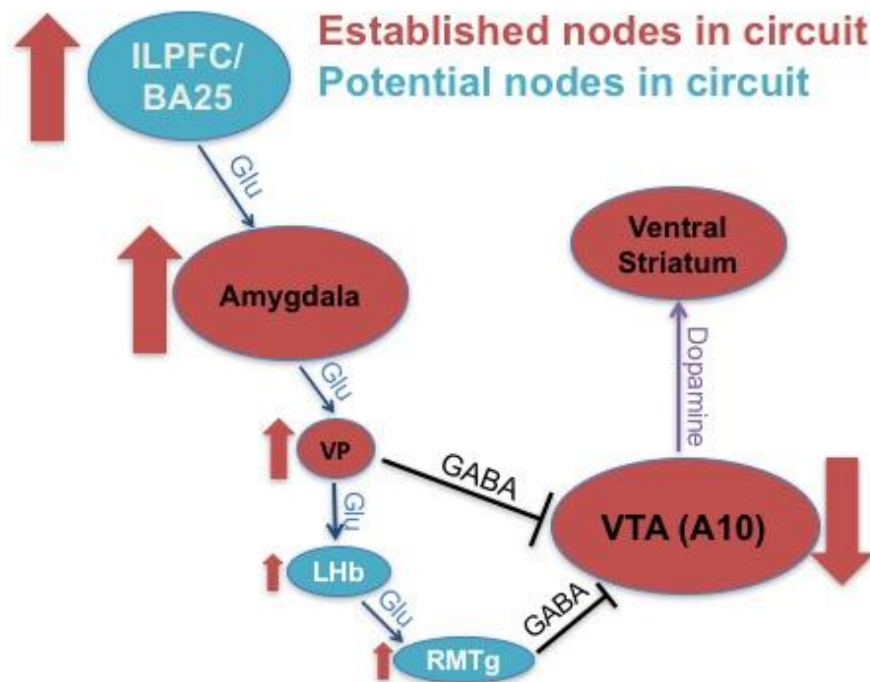


Figure 2. Established and Potential Nodes in CMS Circuit

Chang and Grace (2014) identified the BLA and VP as driving down VTA dopamine neuron population activity following CMS. Other clinically relevant targets including BA25 and LHb have not yet been examined for involvement in this model.

1.3.1 BA25/ILPFC

1.3.1.1 Human Studies

There is strong evidence for altered function of BA25 in MDD based on a large number of brain imaging studies of patients with depression in both resting and active states (Mayberg, 2003).

BA25 activity decreases with depression treatment, and increases with induction of sad mood in healthy individuals or remitted patients (Goldapple et al., 2004; Mayberg et al., 1999). One explanation for this could be that BA25 has decreased volume in familial MDD due to loss of inhibitory neuropil (Ongur, Drevets, & Price, 1998), potentially contributing to the fact that a metabolic increase in this region is seen in MDD patients only after accounting for the decrease in tissue volume. Furthermore, BA25 is also well poised to be part of a network of limbic structures with well documented involvement in MDD such as the amygdala and hippocampus, thus making it an attractive potential target for deep brain stimulation therapy for intractable MDD (Mayberg, 2009).

1.3.1.2 Animal Studies

The rodent homologue of BA25 has been determined to be the ILPFC based on overlapping projections revealed in comparative anatomical study of rodents and nonhuman primates (Heilbronner, Rodriguez-Romaguera, Quirk, Groenewegen, & Haber, 2016). In rodent studies, inhibiting ILPFC astrocytic glutamate uptake induces anhedonia (John et al., 2012). Importantly, activation of the ILPFC in normal animals potently reduces the number of spontaneously active dopamine neurons, in a BLA-dependent manner (Patton et al., 2013). However, this prior study did not examine medial-lateral differences in dopamine neuron inhibition within the VTA, or the contributions of the ILPFC to the reduction in spontaneously active dopamine neurons after CMS.

1.3.2 Lateral Habenula

1.3.2.1 Human Studies

Despite rapidly increasing interest in the region, the LHb at present has comparatively less data available from human studies (Boulos, Darcq, & Kieffer, 2016). Early studies showed that inducing depressive symptoms in remitted major depression patients via tryptophan depletion increased LHb metabolism measured by PET (Morris, Smith, Cowen, Friston, & Dolan, 1999). MR imaging data from the LHb is sparse, attributable to the difficulty with imaging a structure of that size (i.e. less than a few smoothed voxels) and location (i.e. adjacent to pulsatile CSF flow), however modern advances in MR physics and signal acquisition protocols have allowed new studies of this region to be possible (Lawson, Drevets, & Roiser, 2013; Lawson et al., 2014). Data acquired with this new approach in patients with MDD did not show tonic changes in LHb activity, but did reveal diminished responses to aversive stimuli (Lawson et al., 2016). The most compelling clinical evidence for LHb involvement in MDD comes from cases where deep brain stimulation was applied to the lateral habenula with modest improvements in clinical status (Kiening & Sartorius, 2013).

1.3.2.2 Animal Studies

The LHb has potent inhibitory projections to the VTA (Christoph, Leonzio, & Wilcox, 1986) both directly and indirectly through the rostromedial tegmentum (RMTg), which is the major inhibitory relay connecting the LHb with the VTA (Balcita-Pedicino, Omelchenko, Bell, & Sesack, 2011). In studies of awake behaving nonhuman primates, firing of LHb neurons and VTA DA neurons appear to be anticorrelated, with VTA DA neurons firing with receipt of reward and LHb neurons

firing with omission of expected reward, implicating the LHb as a “disappointment signal” (Hikosaka, 2010).

There is a large amount of animal model data on the LHb, primarily using the learned helplessness rodent model of depression. Volumetric increases in the LHb have been observed in rats bred for increased susceptibility to learned helplessness (Henn & Vollmayr, 2005), and increased firing rate has been observed in LHb neurons that project directly to the VTA (B. Li et al., 2011). Reducing afferent drive selectively in the LHb by manipulating protein kinase expression was found to ameliorate helplessness (K. Li et al., 2013).

1.4 PHARMACOLOGICAL TARGETING OF DOPAMINE

As knowledge has increased regarding the alterations in the dopamine system in MDD, so too has interest in exploring the utility of dopaminergic medications for its treatment. Interestingly, amphetamine, which elevates tonic dopamine release via vesicle unloading and reuptake inhibition, has not been found to be significantly helpful as an antidepressant (Satel & Nelson, 1989), other than improving concentration in patients where this is impaired (Reus, Silberman, Post, & Weingartner, 1979). However, the norepinephrine and dopamine reuptake inhibitor (NDRI) bupropion is a very useful antidepressant medication (Zung, 1983), particularly in patients with anhedonia (Nutt et al., 2007), and is approved for use in MDD. Thus, there is a clear role for medications that act on the dopamine system in the treatment of MDD, and greater understanding of the effects of these medications will help guide future efforts to optimize their use in the clinical arena.

1.4.1 Evidence for Quetiapine in MDD

The antidepressant effect of quetiapine was initially identified in studies of its use for schizophrenia (Mullen, Jibson, & Sweitzer, 2001). It was then formally evaluated as monotherapy for bipolar depression in two clinical trials in which it was found to be effective, at lower doses than in schizophrenia (i.e. 300-600 mg/day vs 800 mg/day) (Calabrese et al., 2005; Thase et al., 2006). Quetiapine was next evaluated in patients with unipolar depression, in which it was shown to be effective when used in the augmentation of a SSRI or SNRI (Bauer et al., 2009; McIntyre, Gendron, & McIntyre, 2007) or as monotherapy at 150-300 mg/day (Cutler et al., 2009), with optimal results at 300 mg/day when used as augmentation (El-Khalili et al., 2010) and 150 mg/day when used as monotherapy (Weisler et al., 2009). In addition, maintenance of therapy in patients who experienced remission with quetiapine treatment markedly reduced the risk of depression relapse over the ensuing 1-year follow-up period (Liebowitz et al., 2010).

1.4.2 Antipsychotic drugs effects on dopamine neurons

Upon the discovery that APDs functioned as D2RAs, there was immediate significant interest in identifying the effects of these medications on firing properties of dopamine neurons. There are four decades of history of examining the effects of antipsychotic drugs on the dopamine system.

1.4.2.1 Acute Effects of APDs

The original studies of the effects of D2RAs on dopamine neuron firing properties were performed in the nigra following administration of haloperidol. This resulted in an acute increase in the number of spontaneously active dopamine neurons (Bunney & Grace, 1978). It was determined that there are two predominant mechanisms driving the increase in observed dopamine neuron

activity with APDs (Grace, Bunney, Moore, & Todd, 1997). The first is the blockade of D2 autoreceptors on presynaptic terminals, which is most associated with acute changes in firing rate of dopamine neurons since the somatodendritic D2 autoreceptors are the primary determinant of dopamine neuron firing rate (Bunney & Grace, 1978). The second is the blockade of post-synaptic D2 receptors, and the resultant increase in feedback excitatory drive from the striatum to the VTA via direct and indirect circuits, as lesioning of these feedback pathways prior to D2RA treatment blocks the increase in dopamine neuron population activity (Bunney & Grace, 1978).

The effects of acute treatment with other D2RA agents were found to be similar to haloperidol (Chiodo & Bunney, 1983), with the notable exception that for second generation D2RAs the increase in dopamine neuron population activity was isolated to the A10 (VTA) region, whereas for haloperidol it was observed in both the A9 (nigra) and A10 regions (Skarsfeldt, 1995). The mechanism for this may involve non-dopamine receptor effects of the medications (Chiodo & Bunney, 1985).

1.4.2.2 Chronic Effects of APDs

When quetiapine was administered as repeated treatment for 28 days at a low dose (10 mg/kg), the number of spontaneously active dopamine neurons returned to baseline levels (Skarsfeldt, 1995). However, when administered at higher doses (20-40 mg/kg), quetiapine was found to decrease the number of spontaneously active dopamine neurons (Skarsfeldt, 1995), via induction of depolarization block (Goldstein, Litwin, Sutton, & Malick, 1993).

1.4.2.3 D2RA Treatment in Animal Models of Psychiatric Disorders

It has been observed that the effects of D2RA treatment in an animal model of schizophrenia are different than their effects in normal rats. Specifically, in normal rats, D2RAs require repeated

administration for >21 days in order to induce depolarization block (Bunney & Grace, 1978). However, in the MAM model, D2RAs induce depolarization block after the first dose (Valenti, Cifelli, Gill, & Grace, 2011). Thus there is reason to expect that the effects of D2RA treatment may also differ from that of controls in rats exposed to the CMS model of depression, which involves a markedly hypodopaminergic state. If quetiapine treatment were to normalize dopamine population activity after CMS, this would be expected to contribute to its antidepressant effect.

1.5 GOALS OF DISSERTATION RESEARCH

1.5.1 Circuit Goals

Given the evidence for the involvement of the ILPFC and LHb in depression we sought to characterize the effects on the dopamine system of modulating activity in these regions in normal and CMS-exposed rats. Based on our preliminary data, our hypothesis was that both ILPFC and LHb would inhibit dopamine neuron population activity when activated in normal rats, but that the ILPFC was the primary driver of the CMS-induced deficit in dopamine population activity.

1.5.2 Pharmacology Goals

Given the evidence that D2RAs can increase dopamine population activity we wondered if the documented antidepressant effect of quetiapine could involve a dopaminergic mechanism. Despite extensive literature on the mechanisms of quetiapine's antipsychotic effect in the context of schizophrenia, little is known about its antidepressant mechanism. Furthermore, most existing

theories center not on quetiapine itself, but rather its metabolite N-Desalkyl quetiapine that acts as a reuptake inhibitor of norepinephrine. Because rats do not naturally produce this metabolite (Cross et al., 2016), the isolated effects of the quetiapine could be examined in our rodent model. Thus, we administered quetiapine in acute and repeated dosing to CMS-exposed rats in order to attempt to elucidate a possible contribution of its D2RA properties to its antidepressant mechanism.

2.0 INVOLVEMENT OF INFRALIMBIC PREFRONTAL CORTEX BUT NOT LATERAL HABENULA IN DOPAMINE ATTENUATION AFTER CHRONIC MILD STRESS

Moreines, J.L., Owruksy, Z.L., & Grace, A.A. Select role for infralimbic cortex but not habenula in dopamine attenuation after chronic mild stress. *Neuropsychopharmacology* (In Press)

2.1 INTRODUCTION

Depression has been classically associated with dysfunction within the serotonin system, based primarily on the mode of action of antidepressant drugs (Carlsson, 1976). However, acutely increasing serotonin levels fails to alleviate symptoms, and antidepressant drugs require 8-12 weeks of treatment to be efficacious (Ressler & Nemeroff, 2000). In contrast, dopamine system dysfunction contributes to two key characteristics of depression: anhedonia (Treadway & Zald, 2011) and amotivation (Salamone & Correa, 2012). As a consequence, the dopamine system has garnered increasing attention for its role in depression (Nestler & Carlezon, 2006).

Dopamine neurons can fire in two distinct patterns, a spontaneous irregular tonic pattern and a more selectively recruited phasic burst pattern, the latter proposed to be the salient output of the system signaling behaviorally relevant stimuli (Grace & Bunney, 1984a, 1984b). Because dopamine neurons must be spontaneously active to burst fire, the amount of baseline tonic activity represents the gain, or level of amplification, of the phasic response (Lodge & Grace, 2006a), with tonic firing regulated by a polysynaptic circuit leading to the ventral pallidum (Grace et al., 2007).

In psychiatric disorders, aberrant functioning of this afferent control system is the more common source of dopamine system dysfunction, rather than a primary pathology of the dopamine neurons per se (Grace, 2016).

Using two distinct animal models of depression, we have recently demonstrated deficits in tonic dopamine neuron activity, expressed as reduced population activity of ventral tegmental area (VTA) dopamine neurons (Belujon & Grace, 2014; Chang & Grace, 2014). Moreover, we identified critical involvement of the ventral pallidum and basolateral amygdala in driving the reduced dopamine neuron population activity observed in rats exposed to the chronic mild stress (CMS) rodent depression model (Chang & Grace, 2014).

A recent surge of clinical and translational research has identified multiple additional circuit nodes potentially involved in depression-related dopamine system dysregulation. Brodmann Area 25 (BA25) is homologous with the rodent infralimbic prefrontal cortex (ILPFC) (Heilbronner et al., 2016) and is widely considered to be a critical hub in depression neurocircuitry (Drevets, 1999; Mayberg, 2003). Nearly any form of therapeutically effective treatment for depression is associated with reversal of hypermetabolism in BA25 (Mayberg, 2009). In recent studies of normal rodents, activating ILPFC reduced dopamine neuron activity (Patton et al., 2013) as well as downstream network effects throughout the striatum that correlated with diminished reward-seeking behavior (Ferenczi et al., 2016). However, these experiments did not employ depression animal models.

The lateral habenula (LHb) has also garnered attention for a possible role in depression (Hikosaka, 2010). Clinical data on the LHb are limited owing to its difficulty to image (Lawson et al., 2013); however, it has shown promise as a target for deep brain stimulation for intractable depression (Sartorius et al., 2010). Interest in the region emerged primarily from its role in the

learned helplessness (LH) model of depression (Henn & Vollmayr, 2005; B. Li et al., 2011). When activated acutely, the LHb potently inhibits dopamine neuron firing (Ji & Shepard, 2007) and induces a transient aversive state (Stamatakis & Stuber, 2012) due to reduced synaptic dopamine levels (Hikosaka, Sesack, Lecourtier, & Shepard, 2008). However, the impact of persistent LHb activation on the population activity of dopamine neurons has not been assessed in normal rats or in depression animal models.

In the present study, we characterized the impact of ILPFC and LHb activation on dopamine neuron firing properties in normal rodents, and each region's involvement in the dopamine neuron inhibition induced by CMS. We found that ILPFC and LHb activation have divergent effects on dopamine neuron firing properties. Furthermore, only ILPFC inactivation following CMS was capable of restoring normal dopamine system activity levels.

2.2 METHODS

2.2.1 Subjects

All experiments were performed using adult male Sprague-Dawley rats (Envigo, Indianapolis, Indiana) weighing ≥ 300 g, housed in pairs in a temperature- (22°C) and humidity- (47%) controlled colony room (lights ON 7:00AM-7:00PM) with food and water available ad libitum, and acclimated to the facilities for 7 days. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

2.2.2 CMS Procedure

Animals receiving CMS were single-housed in individual cages within a dedicated room. CMS was delivered as previously described (Chang & Grace, 2014). Stressors – delivered in the home cage – included periodic restricted access to food and water, cage tilt, damp bedding, continuous overnight illumination, intermittent paired housing with an unfamiliar cage-mate, white noise (80-90db), stroboscopic lighting, and predator odor. Each week, 3-4 stressors were delivered in a novel combination.

2.2.3 Behavioral Assays

The full behavioral impact of our CMS procedure has been detailed previously (Chang & Grace, 2014). Thus, for the present study, only behaviors necessary to verify the CMS effect were assessed. Animals housed on a reverse light cycle were weighed weekly. At the conclusion of CMS, animals were tested for immobility on the Forced Swim Test (FST). Due to differences in sensitivity and behavioral profile of different rodent strains, we have found that the sucrose preference test is not a reliable indicator of dopamine system function specifically in Sprague-Dawley rats (Chang & Grace, 2014), unlike other strains such as Lister Hooded, Long-Evans, or Wistar (Tye et al., 2013; Weiss, 1997; Willner, Lappas, Cheeta, & Muscat, 1994; Willner et al., 1992).

2.2.3.1 FST Procedure

The FST was performed following an established protocol (Slattery & Cryan, 2012) over two sequential days during the dark cycle, using plastic cylinder tubes (50 cm height; 20cm diameter)

filled to 30cm with water (23-25°C). On day 1, the rat was placed in the water for 15 minutes in order to eliminate nonspecific behaviors. On day 2 (24hrs later), the rat was placed in the water for 5 minutes, and its behavior was captured by video for offline blinded scoring. The total time the rat spent immobile on day 2 was tabulated as an index of behavioral despair.

2.2.4 Electrophysiological Recordings

2.2.4.1 Surgery

Electrophysiological recordings were performed with light cycle counterbalanced across experimental groups. Rats were anesthetized with chloral hydrate (400mg/kg; i.p.), placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and maintained at 37°C using a thermocouple-controlled heating pad (Fine Science Tools, Foster City, CA). The skull was cleared of skin and fascia, and a partial craniectomy was performed to access the VTA and ILPFC or LHb using the following target coordinates referenced from bregma: VTA -5.3-5.7mm posterior, +0.6-1.0 mm lateral, and -6.5-9.5mm ventral (to brain surface); ILPFC +3.2mm anterior, +0.5mm lateral, and -5.0mm ventral; LHb -3.6mm posterior, +0.8mm lateral, and -5.5mm ventral.

2.2.4.2 Drug Delivery

Microinfusions of drugs were delivered to the ILPFC or LHb using a 28-gauge stainless steel cannula (Plastics One, Roanoke, VA) as described previously (Patton et al., 2013). All drugs were dissolved in Dulbecco's phosphate buffered saline (dPBS; Sigma Aldrich, St. Louis, MO) as vehicle (VEH). N-methyl-d-aspartate (NMDA; Sigma Aldrich, 0.75µg NMDA/0.5µL dPBS) was used for activation, and tetrodotoxin (TTX; Sigma Aldrich, 1µmol/L) was used for inactivation, based on previous data establishing the neural effects of these dosages (Floresco et al., 2001; Patton

et al., 2013). Infusion concentration was consistent for all regions, while volume was adjusted for brain region size (0.5 μ L for ILPFC; 0.25 μ L for LHb) and infused at a rate of 0.25 μ L/min (total infusion time 2 min for ILPFC, 1 min for LHb). The cannula was left in place >5 min following infusion to allow for adequate diffusion.

2.2.4.3 VTA Single Unit Recordings

Twenty minutes following drug microinfusion into LHb or ILPFC, single unit electrophysiological recordings of identified VTA dopamine neurons were performed. Single barrel electrodes were constructed from 2mm diameter borosilicate capillary tubes (World Precision Instruments, Sarasota, FL) using a vertical electrode puller (Narishige, Tokyo, Japan), broken to a target of 6-10M Ω under microscopic control, and filled with 2% Chicago Sky Blue (Sigma Aldrich) dissolved in 2M saline. Signal was acquired using an amplifier (Fintronics, Orange, CT) with open filter settings (50Hz low cutoff, 16kHz high cutoff, 1000x gain) and displayed on an oscilloscope (B&K Precision, Yorba Linda, CA) with signal fed to a computer running Lab Chart 7 (AD Instruments, Sidney, Australia). Units were recorded when signal-to-noise ratio exceeded 3:1.

2.2.4.4 Dopamine Neuron Identification

Electrodes were lowered into the VTA using a manual hydraulic microdrive (Kopf Instruments) and dopamine neurons were identified using well established criteria including location, slow, irregular firing pattern, and long duration, variable shape biphasic action potential waveform (>2.2msec) and half-width (>1.1msec) (Grace & Bunney, 1983a; Ungless & Grace, 2012) (Figure 3). Each dopamine neuron identified was recorded for 3 minutes (1 minute minimum). Three parameters of dopamine neuron firing were calculated: 1) the number of dopamine neurons identified in each track (i.e. population activity averaged over 9 tracks), 2) firing rate, and 3)

proportion of spikes occurring as burst firing (%SIB) in which the burst onset was defined as two spikes with ≤ 80 msec interspike interval and termination by > 160 msec interspike interval (Grace & Bunney, 1984a).

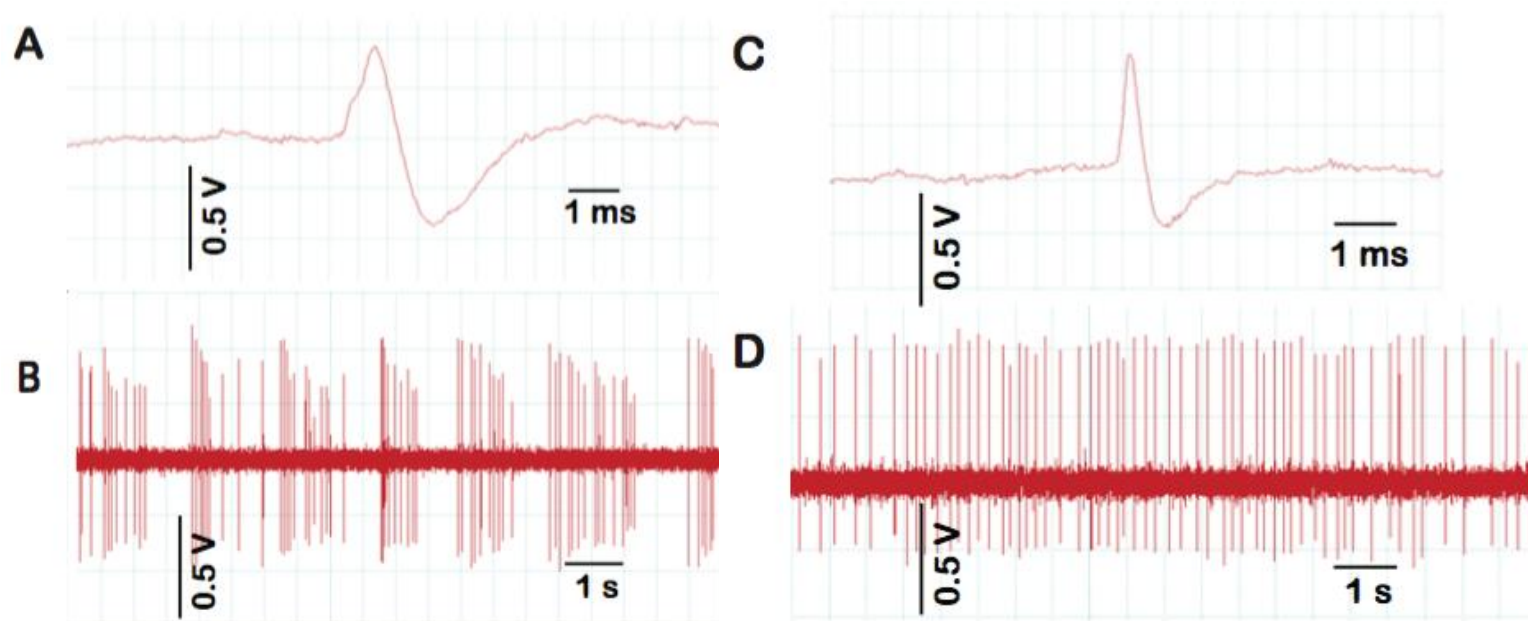


Figure 3. Examples of Neurons Identified as Dopamine and Non-Dopamine

A) Sample trace of dopamine neuron recording demonstrating key action potential features including long duration and biphasic ascending phase. B) Sample recording of VTA dopamine neuron showing characteristic irregular, slow firing rate, mixed with sporadic periods of burst firing. C) Sample trace of a neuron identified as non-dopaminergic. Note the short duration action potential. D) Sample recording of a VTA neuron identified as non-dopaminergic. Note the comparatively more regular firing pattern.

2.2.4.5 VTA Sampling

The VTA was sampled in 9 sequential electrode tracks separated by 0.2mm and arranged in a predetermined grid pattern to assess the distribution of dopamine neuron activity across the medial-lateral extent of the A10 region (Figure 1E). The “Medial” dopamine neurons targeted in this paper include those located in the lateral-most aspects of the Interfascicular (IF) and Central Linear (CL) nuclei and medial aspect of the Paranigral (PN) nucleus. “Central” VTA sampled mostly from the PN nucleus and the medial aspect of the Parabrachial Pigmented (PBP) area. “Lateral” VTA centered on the lateral-most aspects of the PBP area. This procedure was developed to sample dopamine neurons with a variety of different projection targets (Ikemoto, 2007), and has been published by us in multiple prior studies (Valenti, Gill, & Grace, 2012; Valenti, Lodge, et al., 2011). In order for a rat to be included in the final sample, data must have been available from all three VTA locations (i.e. medial, central, and lateral).

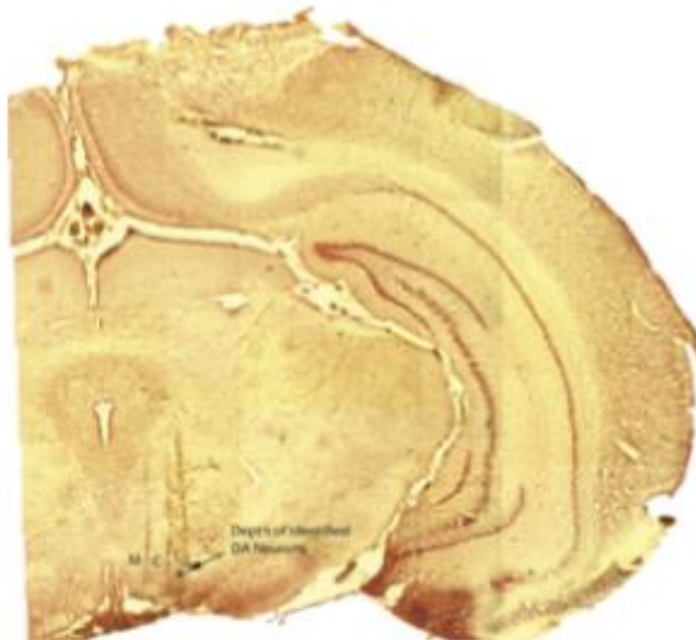


Figure 4. Location of VTA Recording Tracks

Locations of medial (M), central (C), and lateral (L) subregions of VTA. Blue dot in lateral track indicates the depth at which dopamine neurons were found in this animal.

2.2.5 Histology

At the conclusion of recordings, final electrode placement was marked by electrophoretic ejection of dye. Rats were then overdosed with additional chloral hydrate, decapitated, and brains removed. Brains were fixed in 8% paraformaldehyde followed by 25% sucrose for cryoprotection, sectioned using a cryostat (Leica, Buffalo Grove, IL) into 60 micrometer coronal slices, mounted onto gelatin-chromalum coated glass slides, and stained with cresyl violet and neutral red in order to check placements of recording electrodes and infusion cannulae tips. Extent of drug diffusion from ILPFC and LHb microinfusions was estimated from pilot infusions of an identical volume of fluorescent muscimol (TMR-X BODIPY, Fisher, Waltham, MA; Figure 5). To ensure adequate and repeatable VTA sampling across animals, rats were excluded if VTA tracks were: 1) not observed laterally beyond the IF and CL nuclei, 2) located lateral to the PBP (i.e. encroaching on the substantia nigra), or 3) only visible anterior to the PN or posterior to the PBP nucleus.

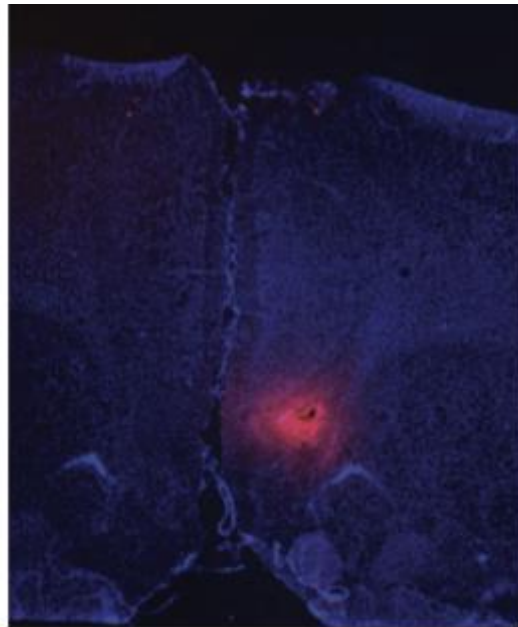


Figure 5. Estimating Diffusion of Infused Drugs

Fluorescent microscope image from infusion of fluorescently tagged marker into ILPFC used to estimate diffusion of subsequent drug microinfusions

2.2.6 Data Analysis

Individual neuron data was analyzed by Lab Chart to identify spike time courses and exported to Neuroexplorer (Nex Technologies, Madison, AL) to calculate firing rate and burst firing. Population activity (i.e. cells per track; CPT) was averaged within each animal followed by across animals in each group, whereas neuron firing rate and burst activity were averaged across animals in a group, considering each cell as an independent replicate. Data were analyzed using SPSS 23 (IBM, Armonk, NY). All data sets were compared to the normal Gaussian distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Data sets that deviated from the normal distribution were analyzed with Mann-Whitney U or Kruskal-Wallis H followed by Dunn's post-hoc tests as appropriate. Normally distributed data were analyzed using independent sample two-tailed t-tests or ANOVA followed by Tukey's post-hoc tests as appropriate.

2.3 RESULTS

2.3.1 Normal Rats

2.3.1.1 ILPFC Activation

ILPFC activation by local infusion of NMDA markedly reduced the number of spontaneously active VTA dopamine neurons recorded per electrode track (VEH 1.1 ± 0.12 CPT, $n=8$; NMDA 0.76 ± 0.038 CPT, $n=11$; $t_{8,4}=2.4$, $p=0.040$; Figure 6A), consistent with prior studies (Patton et al., 2013). ILPFC activation also induced a small but significant increase in average burst firing (VEH 21.6 ± 3.2 %SIB, $n=68$; NMDA 27.7 ± 3.2 %SIB, $n=61$; $U=2.12$, $p=0.034$; Figure 6F). No change

in average firing rate was observed (VEH 3.4 ± 0.26 Hz, $n=68$; NMDA 3.3 ± 0.23 Hz, $n=61$; $U=0.307$, $p=0.76$; Figure 6C).

Given evidence that the VTA is functionally segregated (Ikemoto, 2007), the data were analyzed according to location in medial, central, or lateral VTA. Population activity was profoundly reduced in medial VTA (VEH 1.3 ± 0.12 CPT, $n=8$; NMDA 0.65 ± 0.15 CPT, $n=11$; $t_{17}=3.4$, $p=0.0037$; Figure 6B), but not affected significantly in central and lateral portions, (2way ANOVA Location x Drug interaction, $F_{(2, 34)}=3.35$, $p=0.047$). In contrast, elevated bursting was found to be isolated to neurons in lateral VTA (VEH 20.5 ± 6.7 %SIB, $n=18$; NMDA 39.0 ± 5.4 %SIB, $n=16$; $U=2.48$, $p=0.012$; Figure 6G; Table 1). The frequency distribution of bursting for these neurons suggested that ILPFC activation drove a previously non-bursting population (i.e. zero %SIB) into a higher bursting state (Figure 6H). ILPFC activation did not alter average dopamine neuron firing rate, either overall or in any VTA subregion (Figure 6D; Table 1).

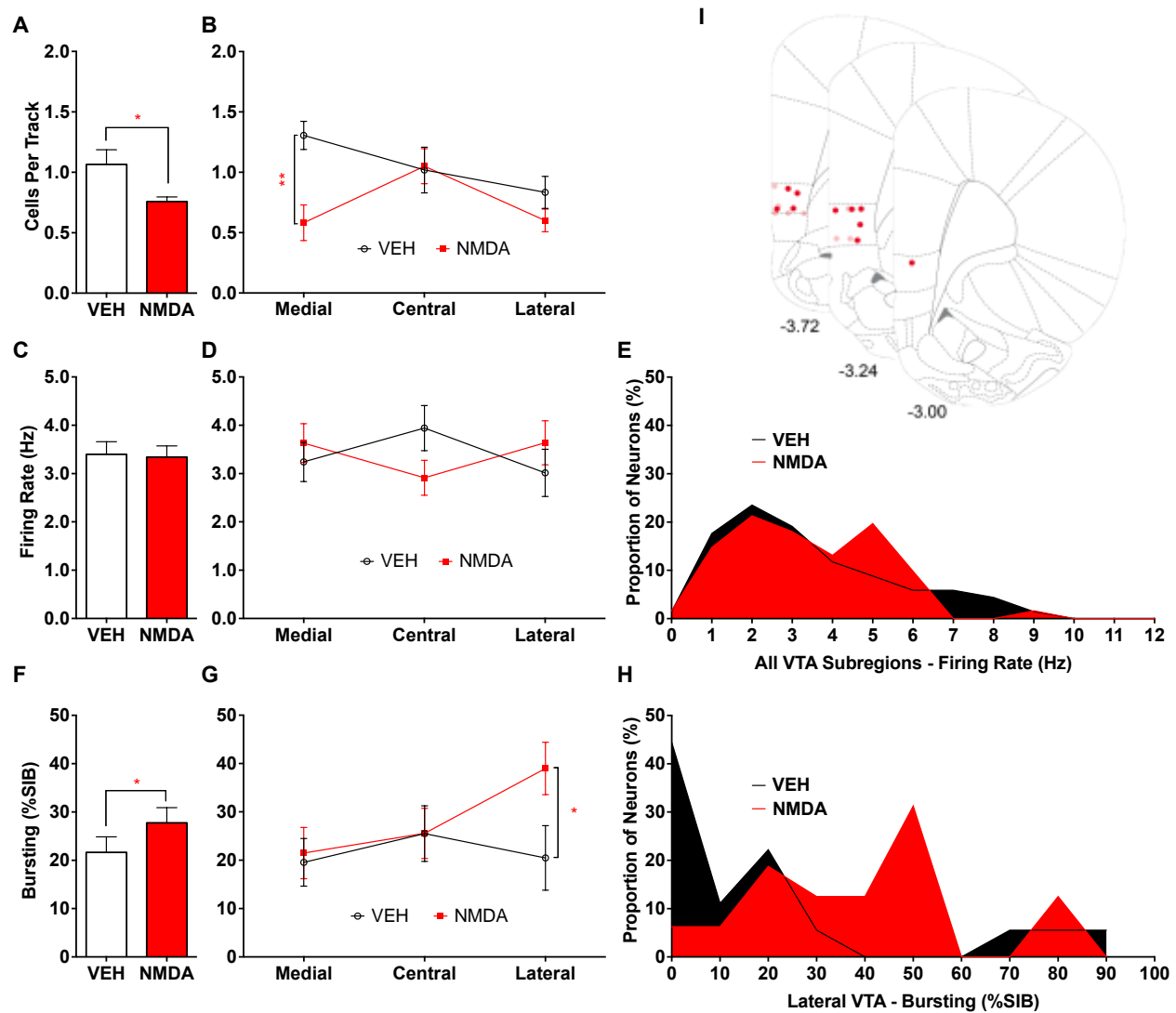


Figure 6. Activation of ILPFC inhibits dopamine population activity

ILPFC activation selectively attenuated dopamine neuron population activity in the medial aspects of the VTA. A) Dopamine neuron population activity was reduced following microinfusion of NMDA into the ILPFC. B) This effect was significant only in medial VTA. C-E) Average firing rate of identified dopamine neurons was not different between animals that received VEH infusions vs. NMDA. F) Average amount of bursting activity in identified dopamine neurons was increased in animals that received ILPFC vs. VEH. G) This occurred via a select increase in bursting activity in dopamine neurons located in lateral VTA. H) Examination of the histograms of lateral VTA dopamine neuron bursting levels for each infusion group suggested that NMDA drove lateral VTA dopamine neurons from a typically non-bursting state (i.e. 0 %SIB) into one in which more neurons showed moderate levels of bursting. I) Location of NMDA (dark red circles) and VEH (light red circles) infusions into ILPFC. * $p < 0.05$; ** $p < 0.01$

Table 1. Summary of Dopamine Neuron Data Following ILPFC Activation

	<u>VEH</u>	<u>NMDA</u>	<u>p-value</u>
Cells Per Track			
All VTA	1.1 ± 0.12	0.76 ± 0.038	t=2.4, p=0.040
Medial	1.3 ± 0.12	0.65 ± 0.15	t=3.4, p=0.0037
Central	0.98 ± 0.21	1.0 ± 0.13	t=-0.28, p=0.78
Lateral	0.85 ± 0.15	0.58 ± 0.088	t=1.7, p=0.11
Firing Rate (Hz)			
All VTA	3.4 ± 0.26	3.3 ± 0.23	U=0.307, p=0.76
Medial	3.2 ± 0.41	3.6 ± 0.40	U=1.3, p=0.18
Central	3.9 ± 0.47	2.9 ± 0.36	U=-1.5, p=0.13
Lateral	3.0 ± 0.49	3.6 ± 0.46	U=1.2, p=0.25
Bursting (%SIB)			
All VTA	21.6 ± 3.2	27.7 ± 3.2	U=2.12, p=0.034
Medial	19.6 ± 4.9	21.5 ± 5.3	U=1.3, p=0.21
Central	25.5 ± 5.7	25.5 ± 5.2	U=0.055, p=0.96
Lateral	20.5 ± 6.7	39.0 ± 5.4	U=2.5, p=0.012
Group Size (Rats)	N=8	N=11	
All VTA (Cells)	N=68	N=61	
Medial (Cells)	N=29	N=20	
Central (Cells)	N=21	N=25	
Lateral (Cells)	N=18	N=16	

2.3.1.2 LHb Activation

Local infusion of NMDA into the LHb caused a marked reduction in dopamine neuron population activity (VEH 1.0 ± 0.087 CPT, $n=10$; NMDA 0.68 ± 0.070 CPT, $n=12$; $t_{20}=3.1$, $p=0.0053$; Figure 7A) along with a modest increase in average VTA dopamine neuron firing rate (VEH 3.3 ± 0.24 Hz, $n=73$; NMDA 4.1 ± 0.25 Hz, $n=69$; $U=2.6$, $p=0.008$; Figure 7C). There was no change in average amount of burst activity (VEH 26.2 ± 3.0 %SIB, $n=73$; NMDA 26.3 ± 2.9 %SIB, $n=69$; $U=0.58$, $p=0.56$; Figure 7F).

In marked contrast to the ILPFC, LHb activation potently and selectively decreased population activity of lateral VTA dopamine neurons, inducing a 70% decrease (VEH 0.88 ± 0.12 CPT, $n=10$, NMDA 0.26 ± 0.063 CPT, $n=12$; $t_{20}=4.9$, $p=0.000092$; Figure 7B). Moreover, LHb activation induced a 39% increase in firing rate of medially located VTA dopamine neurons (VEH 3.3 ± 0.24 Hz, $n=29$; NMDA 4.7 ± 0.36 Hz, $n=32$; $U=2.8$, $p=0.005$; Figure 7D). Bursting was unaffected in all VTA subregions (Figure 7G; Table 2).

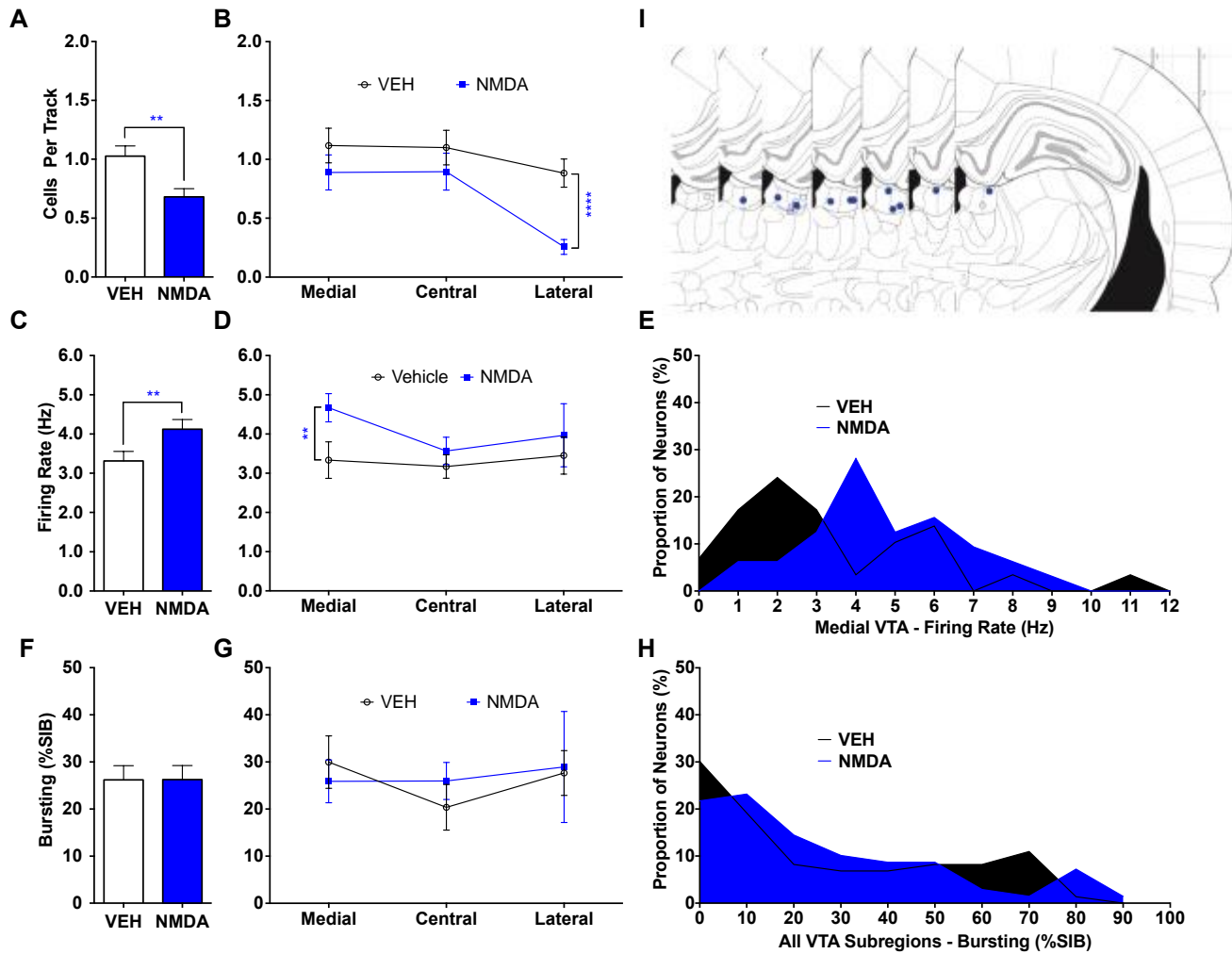


Figure 7. Activation of Lateral Habenula in Normal Rats

LHb activation selectively attenuated dopamine neuron population activity in the lateral aspects of the VTA. A) Dopamine neuron population activity was reduced following infusion of NMDA into the LHb. B) This occurred via selective reduction in lateral VTA dopamine neuron population activity. C) Average firing rate of identified dopamine neurons was higher in NMDA-infused rats vs. VEH. D-E) This effect was driven by a selective increase in the average firing rate of dopamine neurons located in medial VTA following NMDA infusion in LHb. F-H) No changes occurred in average amount of dopamine neuron bursting activity following infusion of NMDA. I) Location of NMDA (closed blue circles) and VEH (open blue circles) infusions into LHb. ** $p < 0.01$; **** $p < 0.0001$

Table 2. Dopamine Neuron Data Following LHb Activation

	<u>VEH</u>	<u>NMDA</u>	<u>p-value</u>
Cells Per Track			
All VTA	1.0 ± 0.087	0.68 ± 0.70	t=3.1, p=0.0053
Medial	1.1 ± 0.15	0.89 ± 0.15	U=-1.2, p=0.25
Central	1.1 ± 0.15	0.90 ± 0.16	U=-0.98, p=0.35
Lateral	0.88 ± 0.12	0.26 ± 0.063	t=-4.9, p=0.000092
Firing Rate (Hz)			
All VTA	3.3 ± 0.24	4.1 ± 0.25	U=2.6, p=0.008
Medial	3.3 ± 0.47	4.7 ± 0.36	U=2.8, p=0.005
Central	3.2 ± 0.30	3.6 ± 0.35	U=0.55, p=0.58
Lateral	3.5 ± 0.47	4.0 ± 0.80	t=-0.58, p=0.57
Bursting (%SIB)			
All VTA	26.2 ± 3.0	26.3 ± 2.9	U=0.58, p=0.56
Medial	30.0 ± 5.6	25.9 ± 4.5	U=0.17, p=0.86
Central	20.4 ± 4.8	26.0 ± 3.9	U=1.4, p=0.16
Lateral	27.7 ± 4.8	28.9 ± 11.8	U=-3.8, p=0.71
Group Size (Rats)	N=10	N=12	
All VTA (Cells)	N=73	N=69	
Medial (Cells)	N=29	N=32	
Central (Cells)	N=24	N=29	
Lateral (Cells)	N=20	N=8	

2.3.2 Chronic Mild Stress

In order to determine the relevance of ILPFC and LHb activity to dopamine system hypofunction in an animal model of depression, the regionally specific effects of CMS on VTA dopamine neuron firing, and how this compares with the impact of ILPFC and LHb activation, were assessed.

2.3.2.1 Vehicle Infused Rats

Following CMS, animals showed increased FST immobility (CON 173.4 ± 23.38 sec, $n=8$, CMS 233.3 ± 9.91 sec, $n=8$, $t_{14}=2.36$, $p=0.037$; figure S1a) and reduced body weight gain (2-way ANOVA Group x Time Interaction $F_{(5, 36)} = 3.7$, $p=0.0078$; figure S1b), consistent with prior studies (Chang & Grace, 2014). CMS-exposed rats exhibited >50% fewer spontaneously active VTA dopamine neurons (CON-VEH 1.3 ± 0.091 CPT, $n=12$, CMS-VEH 0.62 ± 0.049 CPT, $n=14$; $H=22.4$, $p<0.0001$; Figure 8A), with no change in dopamine neuron firing rate (CON-VEH 3.8 ± 0.18 Hz, $n=125$, CMS-VEH 3.7 ± 0.24 Hz, $n=82$; $H=6.0$, $p=0.11$; Figure 8C) or bursting (CON-VEH 31 ± 2.4 %SIB, CMS-VEH 28 ± 2.9 %SIB, $H=2.9$, $p=0.40$; Figure 8F), also consistent with prior studies (Chang & Grace, 2014). When examined with regard to VTA subregions, CMS-exposed rats showed 67% reductions in dopamine neuron population activity in medial VTA (CON-VEH 1.5 ± 0.13 CPT, $n=12$, CMS-VEH 0.55 ± 0.084 CPT, $n=14$; $H=21.3$, $p<0.0001$; Figure 8B) and 50% in central VTA (CON-VEH 1.4 ± 0.17 CPT, $n=12$, CMS-VEH 0.77 ± 0.10 CPT, $n=14$; $H=14.5$, $p=0.012$; Figure 8B), whereas lateral VTA was not significantly impacted (CON-VEH 0.92 ± 0.16 CPT, $n=12$, CMS-VEH 0.53 ± 0.10 CPT, $n=14$; $H=10.2$, $p=0.12$; Figure 8B). No changes were observed following CMS in average dopamine neuron firing rate or bursting activity in any VTA subregion (Figure 8C-H; Table 3).

2.3.2.2 ILPFC or LHb Inactivation

TTX infusion into the ILPFC of CMS-exposed rats also normalized dopamine neuron population activity in medial (CON-VEH 1.5 ± 0.13 CPT, $n=12$, CMS-ILPFC-TTX 1.1 ± 0.14 CPT, $n=10$; $H=6.6$, $p>0.99$) and central (CON-VEH 1.4 ± 0.17 CPT, $n=12$, CMS-ILPFC-TTX 1.1 ± 0.16 CPT, $n=10$; $H=6.2$, $p=0.77$) VTA to levels comparable to control animals (Figure 8B). A concurrent small reduction in average firing rate of medial dopamine neurons was observed (CON-VEH 3.8 ± 0.27 Hz, $n=50$, CMS-ILPFC-TTX 2.6 ± 0.27 Hz, $n=28$; $H=20.8$, $p=0.023$), possibly due to the emergence of a new population of slow-firing dopamine neurons in medial VTA following TTX infusion into the ILPFC (Figure 8C-E). TTX infusion into the LHb exerted no effect on VTA dopamine neuron population activity or firing parameters in CMS-exposed rats in any subregion of the VTA (Figure 8; Table 3). These data demonstrate a critical role for ILPFC in CMS-induced reductions in dopamine neuron population activity.

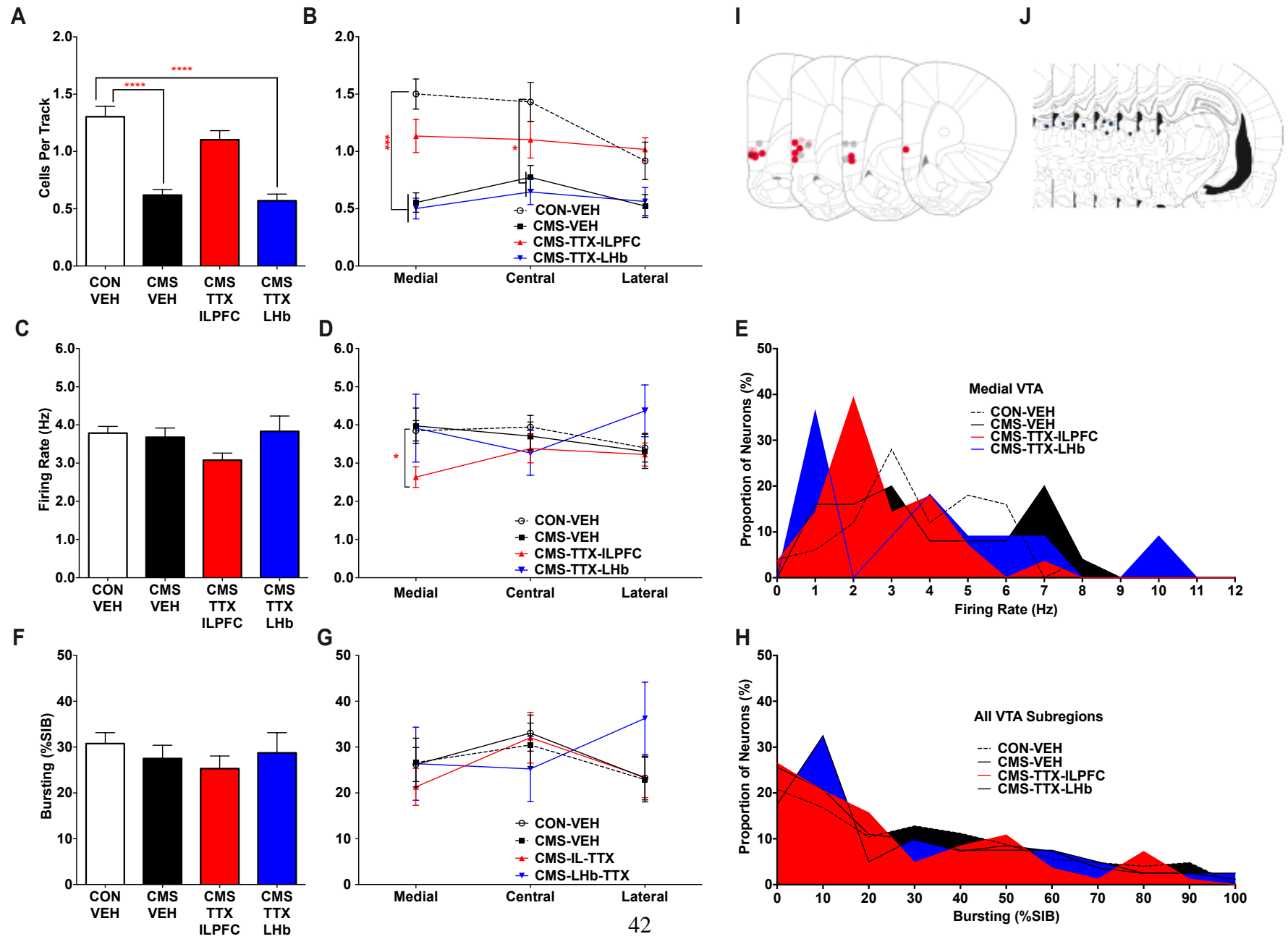


Figure 8. Inactivation of ILPFC or LHb Following Chronic Mild Stress

Only ILPFC inactivation restored VTA dopamine neuron activity in CMS-exposed rats. A) Rats that underwent CMS showed lower VTA dopamine neuron population activity compared to rats housed in CON conditions. Inactivation of ILPFC using microinfusion of TTX restored normal dopamine neuron population activity whereas inactivation of LHb using microinfusion of TTX had no impact on VTA dopamine neuron population activity. B) The reduction in VTA dopamine neuron population activity following CMS was greatest in Medial and Central VTA subregions, while no significant change in dopamine population activity was observed in Lateral VTA. Inactivation of the ILPFC using TTX resulted in normalization of dopamine neuron population activity in Medial and Central VTA subregions. C-D) While VTA-wide average dopamine neuron firing rates did not differ between groups, a select reduction in the average firing rate of dopamine neurons located in medial VTA was observed in CMS-TTX-ILPFC rats compared to CON. E) The histogram of medial VTA dopamine neuron firing rates suggests an increase in the number of dopamine neurons firing at a slower rate following TTX microinfusion into the ILPFC in CMS-exposed rats. F-H) No changes in amount of bursting activity in identified dopamine neurons occurred in any group or VTA subregion. I-J) Location of microinfusions for CON-VEH (grey circles), CMS-VEH (light red and blue circles), CMS-TTX-ILPFC (dark red circles), and CMS-TTX-LHb (dark blue circles). * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$

Table 3. Summary of Dopamine Neuron Data from CMS Experiment

	CON-VEH		CMS-VEH	
	Mean \pm SEM	^a p-value	Mean \pm SEM	^b p-value
Cells Per Track				
All VTA	1.3 \pm 0.091	H=31.3, p<0.0001	0.62 \pm 0.049	H=22.4, p<0.0001
Medial	1.5 \pm 0.13	H=25.4, p<0.0001	0.55 \pm 0.084	H=21.3, p<0.0001
Central	1.4 \pm 0.17	H=13.1, p=0.0045	0.77 \pm 0.10	H=14.5, p=0.012
Lateral	0.92 \pm 0.16	H=10.5, p=0.015	0.53 \pm 0.10	H=10.2, p=0.12
Firing Rate (Hz)				
All VTA	3.8 \pm 0.18	H=6.0, p=0.11	3.7 \pm 0.24	^c -
Medial	3.8 \pm 0.27	H=7.9, p=0.049	4.0 \pm 0.46	H=0.90, p>0.99
Central	3.9 \pm 0.30	H=2.2, p=0.53	3.7 \pm 0.38	^c -
Lateral	3.4 \pm 0.38	H=2.3, p=0.51	3.3 \pm 0.45	^c -
Bursting (%SIB)				
All VTA	30.8 \pm 2.4	H=2.9, p=0.40	27.5 \pm 2.9	^c -
Medial	26.2 \pm 3.7	H=4.3, p=0.23	26.7 \pm 5.3	^c -
Central	33.1 \pm 3.9	H=1.5, p=0.68	30.5 \pm 4.8	^c -
Lateral	23.3 \pm 4.8	H=29, p=0.40	22.9 \pm 4.9	^c -
Group N (Rats)				
All VTA (Cells)	N=12	-	N=14	-
Medial (Cells)	N=125	-	N=82	-
Central (Cells)	N=50	-	N=25	-
Lateral (Cells)	N=47	-	N=34	-
	N=28	-	N=23	-

Table 3 (continued).

CMS-TTX-ILPFC		CMS-TTX-LHb	
Mean \pm SEM	^b p-value	Mean \pm SEM	^b p-value
1.1 \pm 0.078	H=3.9, p>0.99	0.57 \pm 0.057	H=24.4, p<0.0001
1.1 \pm 0.14	H=6.6, p>0.99	0.50 \pm 0.090	H=22.2, p<0.001
1.1 \pm 0.16	H=6.2, p=0.77	0.65 \pm 0.11	H=17.8, p=0.0062
1.0 \pm 0.10	H=-4.7, p>0.99	0.56 \pm 0.12	H=8.8, p=0.38
3.1 \pm 0.18	^c -	3.8 \pm 0.40	^c -
2.6 \pm 0.27	H=20.8, p=0.023	3.9 \pm 0.89	H=4.3, p>0.99
3.4 \pm 0.37	^c -	3.3 \pm 0.59	^c -
3.2 \pm 0.30	^c -	4.4 \pm 0.68	^c -
25.3 \pm 2.8	^c -	28.7 \pm 4.4	^c -
21.3 \pm 4.0	^c -	26.4 \pm 8.0	^c -
32.1 \pm 5.5	^c -	25.3 \pm 7.1	^c -
23.5 \pm 4.5	^c -	36.3 \pm 7.9	^c -
N=10	-	N=8	-
N=83	-	N=40	-
N=28	-	N=11	-
N=28	-	N=15	-
N=27	-	N=14	-

^aKruskal-Wallis test for all groups;

^bDunn's post-hoc test vs. CON-VEH group;

^cNo post-hoc performed because Kruskal-Wallis p>0.05

2.4 DISCUSSION

In this study, activation of either the ILPFC or the LHb in normal animals reduced the population activity of VTA dopamine neurons (i.e. the number of dopamine neurons spontaneously active). Whereas ILPFC activation exerted a greater inhibitory effect on medial VTA dopamine neurons, the LHb selectively inhibited lateral VTA dopamine neurons (Figure 9). In rats exposed to CMS, the reduction in dopamine neuron population activity was preferential to medial and central VTA. ILPFC inactivation restored dopamine neuron population activity to a normal level, whereas LHb inactivation did not affect this parameter.

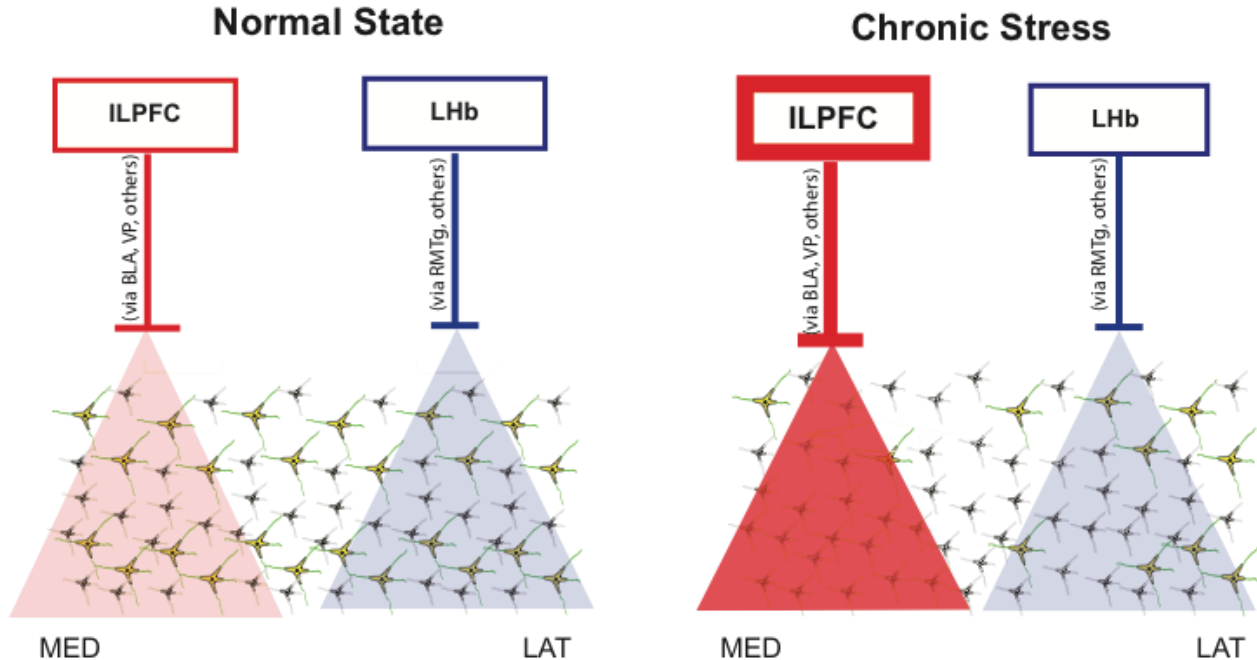


Figure 9. One Potential Circuit Diagram Proposed to Account for ILPFC and LHb Regulation of Dopamine Neuron Population Activity in Normal and Stressed States

In the normal state, afferent regions are functioning at baseline levels, leading to baseline levels of VTA dopamine neuron population activity. When activated, the ILPFC preferentially inhibits dopamine neurons located in medial VTA, which tend to project more to the reward- and motivation-related areas of the nucleus accumbens, whereas the LHb preferentially inhibits dopamine neurons located in lateral VTA, which project more to cognitive/decision related dorsomedial striatal regions [See Ikemoto (2007), Figure 12 for review of anatomy]. CMS involves ILPFC-driven inhibition of dopamine neurons, as its inactivation restored normal levels of dopamine neuron population activity

Following some of the manipulations, in addition to changes in population activity, there were also small but significant changes in firing rate and bursting. We have reported previously that DA neurons must be spontaneously active to respond to behaviorally relevant, phasic inputs (Floresco et al., 2003; Lodge & Grace, 2006a). Therefore, a change in population activity better reflects the responsivity of the system, and is altered in several disease states (Grace, 2016). ILPFC activation in normal rats reduced dopamine population activity medially but increased the tendency of lateral neurons to burst fire. Because the majority of dopamine neurons in medial VTA project to accumbens shell, reducing the active number would be expected to markedly reduce the response of ventromedial accumbens-projecting DA neurons to reward-related stimuli (Grace, 2016) since only spontaneously active dopamine neurons are capable of responding to signals with burst firing. This may underlie the reduced motivation for reward in depressed individuals, as Ferenczi et al (2016) showed activation of the ILPFC induced depression-related behavior in normal rats that was associated with functional connectivity changes between the ILPFC and ventral striatum. In contrast, the increased tendency to burst fire was observed in lateral VTA dopamine neurons, which project more strongly to associative striatum, although the number of spontaneously active neurons did not change. Although speculative, this tonic change in bursting could reflect changes in plasticity with respect to the salience of environmental stimuli, contributing to the well-documented affective bias and hypersensitivity to negative valence stimuli and feedback in depression (Clark, Chamberlain, & Sahakian, 2009).

Following CMS, inactivation of the ILPFC restored the number of spontaneously active dopamine neurons to normal levels, while reducing the average firing rate of medial dopamine neurons, due to an increase in the number of slower firing dopamine neurons (Figure 8E). While

changes in firing rate may engender a change in baseline DA levels in the accumbens, the impact on responsivity to stimuli would be comparatively minimal and likely to undergo homeostatic compensation.

Intriguingly, ILPFC inactivation in CMS-exposed rats restored normal dopamine population activity in both medial and central VTA, while ILPFC activation in normal rats inhibited only medial VTA population activity. In normal rats, ILPFC inactivation increases dopamine neuron population activity through indirect activation of the ventral subiculum of the hippocampus via the nucleus reunions (Patton et al., 2013; Zimmerman & Grace, 2016). Thus, inhibition of ILPFC in the CMS-exposed rats in the present paper (or inhibition of BA25 in patients with depression such as via DBS) may engage distant downstream brain networks that together work to restore normal dopamine-mediated hedonic functions via a mechanism that may differ from simple reversal of abnormal plasticity per se.

LHb activation in normal rats reduced dopamine population activity laterally but increased firing rate medially. Inhibition of lateral dopamine neurons has been shown to alter reward expectation and decision-making (Eshel, Tian, Bukwich, & Uchida, 2016), supporting a role for LHb-mediated inhibition of these neurons impacting cognitive tasks (Stopper & Floresco, 2014). Conversely, the small increase in medial dopamine neuron firing rate with LHb activation likely reflects a known, small, monosynaptic glutamatergic projection from the LHb to cortically projecting dopamine neurons in medial VTA (Lammel et al., 2012). Although this di-synaptic projection was shown to be aversive functionally (assessed by conditioned place avoidance), increased activity of cortically-projecting dopamine neurons has actually been shown to be protective against developing depression (Chaudhury et al., 2013). In CMS-exposed rats, LHb inactivation had no effect. Importantly, LHb activation has previously been shown to be aversive

(Stamatakis & Stuber, 2012), but never to be pro-depressive in normal animals (i.e. without a genetic predisposition to helplessness). Moreover, recent neuroimaging data questions whether heightened LHB activity actually drives responses in depressed humans (Lawson et al., 2016).

These results add to the accumulating evidence supporting a major role of the dopamine system in stress and depression. Dopamine lesions, antagonists, or direct optogenetic attenuation of dopamine neurons reduces reward pursuit similar to that seen in depressed patients with anhedonia, whereas rats that have received CMS show a reversal of behavioral despair by optogenetic activation of VTA dopamine neurons (Tye et al., 2013). While this may at first appear inconsistent with studies using the chronic social defeat (CSD) model, in which dopamine system activation facilitates defeat-mediated depression (Chaudhury et al., 2013), this may have more to do with the relative timing of the interventions. Thus, in each of the studies with CMS or LH, dopamine neuron activity was measured >24 hours after removal of the stressor. In contrast, activation of accumbal-projecting dopamine neurons with social defeat during the induction phase increased the susceptibility of mice to defeat-induced depression, consistent with an established role for these neurons in acute stress (Valenti, Lodge, et al., 2011). These collective data are highly consistent with a model in which stress-induced dopamine neuron activation is followed by a much longer attenuation of dopamine neuron activity, as has been observed 24 hours after dopamine system activation by restraint (Chang & Grace, 2013) or amphetamine (Belujon et al., 2016), and following chronic cold stress (Moore, Rose, & Grace, 2001; Valenti et al., 2012). This is consistent with Koob's opponent process model, in which dopamine activation is followed by a long-duration compensatory attenuation of the dopamine system (Koob, Stinus, Le Moal, & Bloom, 1989). Thus, we observed the decrease in dopamine neuron activity following withdrawal of LH or CMS (Belujon & Grace, 2014; Chang & Grace, 2014), and Tye et al (2013) found that VTA dopamine

neuron activation reversed the impact of CMS following withdrawal of the stressor, which was later replicated in the CSD depression model as well (Friedman et al., 2014).

In summary, the current data highlight distinct roles for the ILPFC and LHb in regulating VTA dopamine neurons in normal and chronic stress-exposed states. If this translates to major depressive disorder in humans, this also would provide a target site for therapeutic intervention within the ILPFC/BA25 to normalize dopamine neuron drive.

3.0 DIVERGENT EFFECTS OF ACUTE AND REPEATED QUETIAPINE TREATMENT ON DOPAMINE SYSTEM IN NORMAL AND CHRONIC MILD STRESS INDUCED HYPODOPAMINERGIC STATES

Moreines JL, Owruksy ZL, Gagnon, KG, Grace AA. Divergent Effects of Acute and Repeated Quetiapine Treatment on Dopamine System in Normal and Chronic Mild Stress Induced Hypodopaminergic States. In Preparation.

3.1 INTRODUCTION

Quetiapine and other second-generation dopamine (DA) D2 receptor antagonists (D2RA), e.g. lurasidone, are increasingly popular monotherapy and as an adjunct to antidepressant drugs in patients with inadequate responses to traditional antidepressants alone (Saunders & Goodwin, 2013). However, the therapeutic mechanism of these agents in depression is largely unknown. For quetiapine specifically, existing theories highlight the role of a drug metabolite N-Desalkyl quetiapine, which functions as a potent norepinephrine reuptake inhibitor (Chernoloz, El Mansari, & Blier, 2012). However, this model does not incorporate the D2RA properties of quetiapine.

Mounting evidence suggests that in depression the dopamine system is underactive (Belujon & Grace, 2015; Grace, 2016). Thus, it is important to reconcile how a compound that is thought to block dopamine transmission may actually help a condition that results from already reduced dopamine system function. Importantly, existing theories are based on data from administering quetiapine to normal rats, whereas the effect of quetiapine on the dopamine system

in rodent depression models has not been examined. However, as has become clear in other settings such as animal models of schizophrenia, the effects of D2RAs on a normally functioning dopamine system often differ substantially from their effects on a dopamine system functioning in a pathological state (Valenti, Cifelli, et al., 2011). It is therefore critical to examine the impact of quetiapine on animal models of depression that exhibit clinically relevant pathological functioning of the dopamine system.

Dopamine neurons fire in two patterns, spontaneous firing and burst firing (Grace et al., 2007). In order to burst fire a neuron must already be spontaneously active. We have recently reported that across multiple animal models of depression, including chronic mild stress (CMS) (Moreines, Owruksy, & Grace, 2016) and learned helplessness (Belujon & Grace, 2014), the number of spontaneously active dopamine neurons is markedly reduced without changes to their firing rate or amount of burst firing. In the CMS model specifically, we have further elucidated that this decrease in dopamine population activity following exposure to chronic stress is due to enhanced afferent GABA-ergic input from the ventral pallidum driving dopamine neurons into a silent state, as inactivation of this region restores dopamine population activity to normal levels (Chang & Grace, 2014).

Multiple studies across animal models (Tye et al., 2013) and human patients with depression (Schlaepfer, Bewernick, Kayser, Madler, & Coenen, 2013) have suggested that restoring normal dopamine system function could be a valuable approach to treating depression, particularly in cases with prominent dopamine related symptoms such as anhedonia. Thus, if quetiapine were to increase the population activity of dopamine neurons, this would be expected to contribute to its antidepressant effect. While the effects of quetiapine on the dopamine system have been extensively studied in normal rodents, the effects of quetiapine on the dopamine system

in animal models of depression has not been studied. Specifically, acute administration of quetiapine at antidepressant doses has been shown to increase dopamine population activity (Goldstein et al., 1993), whereas after repeated administration dopamine neuron population activity is at baseline or lower levels (Skarsfeldt, 1995). However, behavioral studies using the CMS model suggest that quetiapine may have the ability to reverse deficits in behavioral assays relevant to depression, specifically when administered at the lower antidepressant-level dose of 10 mg/kg for long durations (Orsetti et al., 2007). This suggests that the effects of repeated quetiapine may differ in the presence of factors driving a hypodopaminergic state, e.g. chronic stress.

In the present study, we evaluated the acute and repeated treatment effects of an antidepressant dosage of quetiapine in both normal and CMS-exposed rats. We find that repeated quetiapine administration produces strikingly different effects in normal vs. stressed rodents.

3.2 METHODS

3.2.1 Subjects

All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Adult male Sprague-Dawley rats (Envigo, Indianapolis, Indiana) weighing ≥ 300 g were used for all experiments. They were acclimated to the facilities for >7 days, housed in pairs in a temperature- (22°C) and humidity- (47%) controlled colony room (lights ON 7:00AM-7:00PM). Food and water were available ad libitum.

3.2.2 CMS Procedure

CMS was performed as reported previously (Chang & Grace, 2014; Moreines et al., 2016). CMS exposed rats were single-housed in individual cages within an isolated colony room. Stressors included periodic restricted access to food and water, cage tilt, damp bedding, continuous overnight illumination, intermittent paired housing with an unfamiliar cage-mate, white noise (80-90db), stroboscopic lighting, and predator odor, all delivered in the home cage. Each week, 3-4 stressors were delivered in a novel combination.

3.2.3 Drug Preparation

Quetiapine 10 mg/mL was dissolved in 0.3% tartaric acid in physiological saline and neutralized with sodium hydroxide to a final pH of 5-6. This dose (10 mg/kg) was selected based on prior studies showing electrophysiological (Goldstein et al., 1993) and behavioral (Orsetti et al., 2007) effects at this dose. Vehicle treated rats received 1 ml/kg injections of the same solution without the addition of quetiapine; the two solutions were equivalent in pH and temperature.

3.2.4 Electrophysiological Recordings

3.2.4.1 Surgery

Electrophysiological recordings were performed during the light phase. Rats anesthetized with chloral hydrate (400mg/kg; i.p.) were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and maintained at 37°C using a thermocouple-controlled heating pad (Fine Science

Tools, Foster City, CA). To access the VTA, the skull was cleared of skin and fascia, and a partial craniectomy of the skull was performed 5.3-5.7mm posterior and +0.6-1.0 mm lateral to bregma.

3.2.4.2 Signal Acquisition

Single barrel electrodes were constructed with a vertical electrode puller (Narishige, Tokyo, Japan) using 2mm diameter borosilicate capillary tubes (World Precision Instruments, Sarasota, FL), and then broken to a target of 6-10M Ω under microscopic control and filled with a 2M saline solution with 2% Chicago Sky Blue (Sigma Aldrich). Signals were fed through silver wire to an amplifier (Fintronics, Orange, CT) operated with open filter settings (50Hz low cutoff, 16kHz high cutoff, 1000x gain), displayed on an oscilloscope (B&K Precision, Yorba Linda, CA), and stored on a computer running Lab Chart 7 (AD Instruments, Sidney, Australia). Units were recorded when signal-to-noise ratio exceeded 3:1.

3.2.4.3 VTA Sampling and Dopamine Neuron Identification

The VTA was sampled over a grid of 9 sequential electrode tracks separated by 0.2mm and arranged in a predetermined pattern to reliably sample across the medial-lateral and anterior-posterior extent of the A10 region. Electrodes were lowered into the VTA using a manual hydraulic microdrive (Kopf Instruments) and dopamine neurons were identified using well established criteria including location, slow, irregular firing pattern, and long duration, variable shape biphasic action potential waveform (>2.2msec) and half-width (>1.1msec) (Grace & Bunney, 1983a; Ungless & Grace, 2012). Identified dopamine neurons were recorded for 3 minutes (1 minute minimum), and three parameters of dopamine neuron firing were calculated: 1) the number of spontaneously firing dopamine neurons identified in each track (i.e. population activity averaged over 9 tracks), 2) firing rate, and 3) proportion of spikes occurring as burst firing (%SIB) in which

the burst onset was defined as two spikes with ≤ 80 msec interspike interval and termination by > 160 msec interspike interval (Grace & Bunney, 1984a).

3.2.5 Experimental Outline

The experiments performed are outlined in Figure 10.

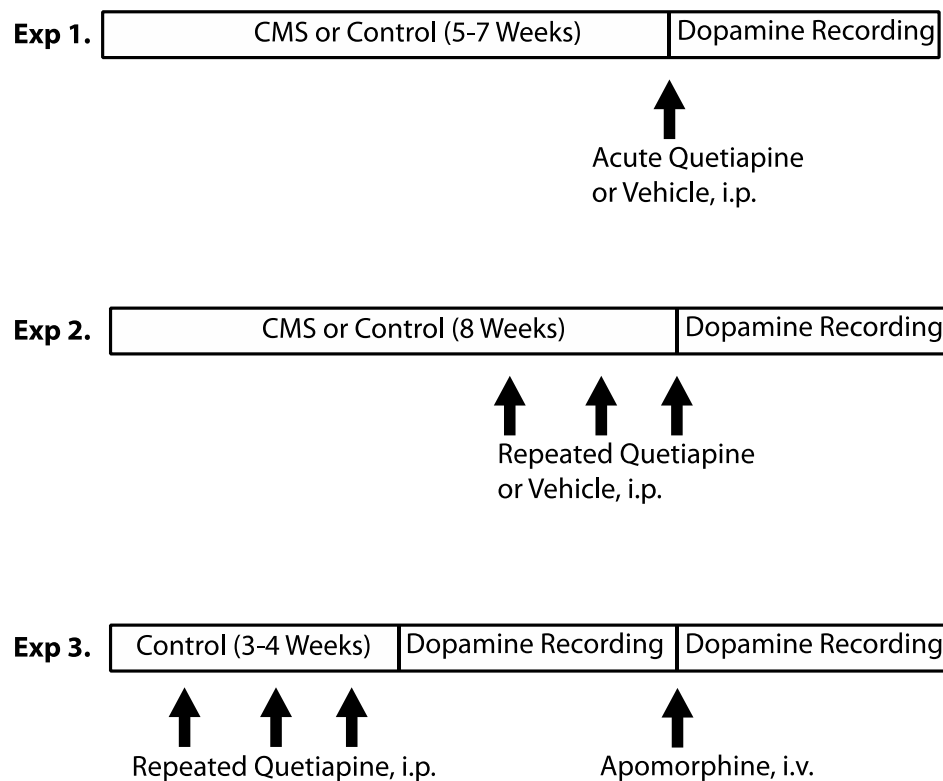


Figure 10. Experimental Timeline of CMS QTP

In Experiments 1 and 2, rats experienced 5-7 weeks of CMS or control conditions. This was followed by acute administration of quetiapine on the day of dopamine neuron recording (Experiment 1) or 21+ days of quetiapine up to the day of dopamine neuron recording (Experiment 2). In Experiment 3, rats living in normal housing conditions received 21+ days of quetiapine up to the day of dopamine neuron recording, which included measurements of dopamine neuron population activity before and immediately following a pre-synaptic dose of apomorphine.

3.2.5.1 Acute quetiapine in normal and CMS-exposed rats

Rats were exposed to 5-7 weeks of CMS or control conditions. On the day of dopamine neuron recording, quetiapine was administered 2 hours prior to beginning the recording.

3.2.5.2 Repeated quetiapine in normal and CMS-exposed rats

Rats were exposed to 5 weeks of standard CMS or control conditions, followed by an additional 3+ weeks during which they received daily injections of quetiapine 10 mg/kg or vehicle in parallel with the CMS protocol. Electrophysiological recordings began 2 hours following the final dose.

3.2.5.3 Repeated quetiapine plus apomorphine in normal rats

Rats received daily injections of quetiapine 10 mg/kg for >21 days, which is a standard minimum duration for the induction of depolarization block (Grace et al., 1997). Electrophysiological recordings were performed beginning 2 hours after the final dose. A recording of VTA population activity was performed. Apomorphine dissolved in normal saline (50 mcg/mL) was then delivered intravenously at autoreceptor-selective dosages via the lateral tail vein until a decrease in firing rate of >20% was observed, with most rats showing response with 40-80 µg/kg, consistent with prior studies (Valenti, Cifelli, et al., 2011). The VTA was then resampled for population activity. These recordings were counterbalanced across animals for anteroposterior starting location within the VTA.

3.2.6 Histology

Final electrode placement at the conclusion of recording was marked by electrophoretic ejection of Pontamine dye. Rats were overdosed with additional chloral hydrate and decapitated. Brains were removed and fixed in 8% paraformaldehyde followed by 25% sucrose for cryoprotection. Sixty micrometer coronal sections were prepared using a cryostat (Leica, Buffalo Grove, IL), mounted onto gelatin-chromalum coated glass slides, and stained with cresyl violet and neutral red for microscopic confirmation of recording electrode location.

3.2.7 Data Analysis

Spike time courses for individual neurons were exported from Lab Chart into Neuroexplorer (Nex Technologies, Madison, AL) for calculation of firing rate and burst firing. Within each rat, population activity was calculated by dividing the total number of dopamine neurons identified by the number of valid tracks performed, yielding the metric of Cells Per Track (CPT). The average firing rate and amount of burst activity for dopamine neurons recorded were averaged across rats in a group, considering each cell as an independent replicate. Data were analyzed in SPSS 23 (IBM, Armonk, NY) and Prism 7 (GraphPad, La Jolla, CA). For experiments 1 and 2, 2-way ANOVAs with post-hoc tests were performed to compare treatment groups. For experiment 3, paired t-tests were performed to compare data collected before and after apomorphine administration.

3.3 RESULTS

3.3.1 Experiment 1: Acute quetiapine increased dopamine neuron population activity in normal rats but not chronically stressed rats

The differential effect of acute quetiapine in normal and stress exposed rats was evaluated by administering an acute dose of quetiapine (10 mg/kg) 2 hours prior to assessing VTA dopamine neuron firing properties. This resulted in a significant interaction effect between treatment with acute quetiapine and exposure to CMS for dopamine population activity (2-way ANOVA interaction $F_{(1,25)}=4.3$, $p=0.049$, Figure 11, Table 4). Acute administration of quetiapine to normal rats increased dopamine population activity (CON-VEH-Acute 1.1 ± 0.11 CPT, $n=8$; CON-QTP-Acute 1.4 ± 0.068 CPT, $n=11$; 2-way ANOVA interaction $F_{(1,25)}=4.3$, $p=0.049$; post-hoc $t_{25}=3.3$, $p=0.0054$; Figure 11A, Table 4), in line with prior reported results (Goldstein et al., 1993). There was no change in the average firing rate of recorded dopamine neurons (CON-VEH-Acute 4.4 ± 0.30 Hz, $n=69$; CON-QTP-Acute 3.5 ± 0.23 Hz, $n=120$; 2-way ANOVA $p>0.05$ for main effects of drug or interaction; Figure 11B-D, Table 4) or proportion of burst activity (CON-VEH-Acute 33.9 ± 3.6 %SIB, $n=69$; CON-QTP-Acute 25.0 ± 2.4 %SIB, $n=120$; 2-way ANOVA $p>0.05$ for main effects of drug or interaction; Figure 11E-G, Table 4).

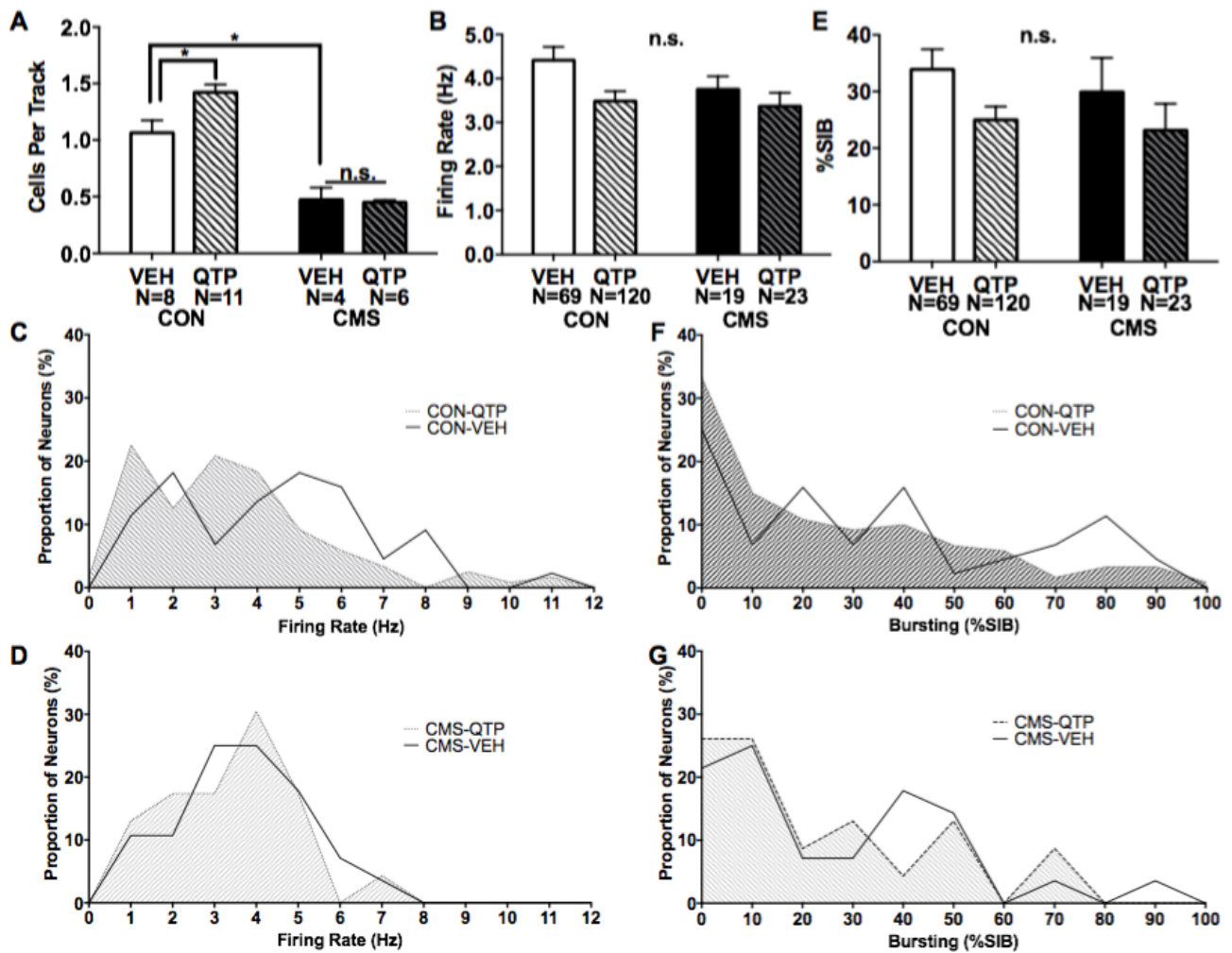


Figure 11. Impact of Acute Quetiapine Treatment in Normal and Chronically Stressed Rats

Acute administration of quetiapine increased dopamine population activity in normal rats, but had no effects on CMS-exposed rats. A) Dopamine population activity was increased following acute administration of quetiapine to normal rats, but the reduction observed in stressed rats was not ameliorated by acute administration. B-G) Average dopamine neuron firing rate (B-D) and proportion of burst firing (E-G) were unchanged by acute administration of quetiapine to normal or CMS-exposed rats.

Table 4. Summary of Dopamine Neuron Data for Acute Quetiapine

	<u>CON-VEH-Acute</u>	<u>CON-QTP-Acute</u>	<u>CMS-VEH-Acute</u>	<u>CMS-QTP-Acute</u>
Cells Per Track	1.1 ± 0.11	1.4 ± 0.068	0.47 ± 0.11	0.45 ± 0.019
Interaction	F _(1,25) = 4.3 ; p = 0.049 ^a	-	-	-
Drug	F _(1,25) = 3.3; p = 0.081 ^a	t ₍₂₅₎ = 3.3; p = 0.0054 ^b	-	t ₍₂₅₎ = 0.15; p = 0.99 ^b
Stress	F _(1,25) = 72.6; p < 0.0001 ^a	-	t ₍₂₅₎ = 4.2; p = 0.0006 ^c	t ₍₂₅₎ = 8.3; p < 0.0001 ^c
Firing Rate (Hz)	4.4 ± 0.30	3.5 ± 0.23	3.7 ± 0.30	3.4 ± 0.31
Interaction	F _(1,227) = 0.46 ; p = 0.50 ^a	-	-	-
Drug	F _(1,227) = 2.6; p = 0.11 ^a	- ^d	-	- ^d
Stress	F _(1,227) = 0.94; p = 0.33 ^a	-	- ^d	- ^d
Bursting (%SIB)	33.9 ± 3.6	25.0 ± 2.4	29.9 ± 6.1	23.2 ± 4.7
Interaction	F _(1,227) = 0.056 ; p = 0.81 ^a	-	-	-
Drug	F _(1,227) = 2.8; p = 0.094 ^a	- ^d	-	- ^d
Stress	F _(1,227) = 0.39; p = 0.53 ^a	-	- ^d	- ^d
Group N (Rats)	N = 8 rats, 69 cells	N = 11 rats, 120 cells	N = 4 rats, 19 cells	N = 6 rats, 23 cells

^aMain effects among all groups;^bSidak's post-hoc test for drug effect (within same stress category);^cSidak's post-hoc test for stress effect (within same drug group);^dMain effect not significant, post-hoc test not performed

In line with prior results, CMS-exposed rats treated acutely with vehicle had fewer spontaneously active dopamine neurons as compared to control rats treated acutely with vehicle (CON-VEH-Acute 1.1 ± 0.11 CPT, $n=8$; CMS-VEH-Acute 0.47 ± 0.11 CPT, $n=4$; 2-way ANOVA main effect of stress $F_{(1,25)}=72.6$; $p < 0.0001$; post-hoc $t_{25}=4.2$, $p=0.0006$; Figure 11A, Table 4), without a change in firing rate (CON-VEH-Acute 4.4 ± 0.30 Hz, $n=69$; CMS-VEH-Acute 3.7 ± 0.30 Hz, $n=19$; 2-way ANOVA $p>0.05$ for main effects of stress or interaction; Figure 11B-D, Table 4) or proportion of burst activity (CON-VEH-Acute 33.9 ± 3.6 %SIB, $n=69$; CMS-VEH-Acute 29.9 ± 6.1 %SIB, $n=19$; 2-way ANOVA $p>0.05$ for main effects of stress or interaction; Figure 11E-G, Table 4).

In contrast to the effect observed in normal rats, quetiapine had no effect on dopamine neuron population activity in CMS-exposed rats (CMS-VEH-Acute 0.47 ± 0.11 CPT, $n=6$; CMS-QTP-Acute 0.45 ± 0.019 CPT; post-hoc $t_{25}=0.15$, $p=0.99$; Figure 11A, Table 4). Similarly, there was no effect on average dopamine neuron firing rate (CMS-VEH-Acute 3.7 ± 0.30 Hz, $n=19$; CMS-QTP-Acute 3.4 ± 0.31 Hz, $n=23$; 2-way ANOVA $p>0.05$; Figure 11B-D, Table 4), or proportion of burst activity (CMS-VEH-Acute 29.9 ± 6.1 %SIB, $n=19$; CMS-QTP-Acute 23.2 ± 4.7 %SIB, $n=23$; 2-way ANOVA $p>0.05$; Figure 11E-G, Table 4).

3.3.2 Repeated Quetiapine Alters Dopamine Activity in Stressed Rats but not Normal Rats

We next assessed whether repeated quetiapine administration differentially affected dopamine neuron activity in normal and CMS-exposed rats. As with acute administration of quetiapine, we observed a significant interaction between CMS exposure and repeated treatment with quetiapine ($F_{(1,42)}=5.2$, $p=0.028$; Figure 12, Table 5). However, in contrast to the effects of acute treatment, repeated quetiapine treatment did not alter dopamine neuron population activity at these doses (CON-VEH-Repeated 0.99 ± 0.06 CPT, $n=10$; CON-QTP-Repeated 0.92 ± 0.10 CPT, $n=13$; $t_{42}=0.53$, $p=0.84$; Table 5A, Figure 12). Similarly, no effect was observed in average dopamine firing rate (CON-VEH-Repeated 2.9 ± 0.21 Hz, $n=78$; CON-QTP-Repeated 3.4 ± 0.21 Hz, $n=92$; 2-way ANOVA $p>0.05$ for main effects of drug or interaction; Figure 12B-D, Table 5). While a main effect of repeated quetiapine on proportion of burst activity was observed across all groups ($F_{(1,301)}=5.4$; $p=0.020$), a post-hoc test within normal rats did not reveal a significant effect (CON-VEH-Repeated 31.6 ± 2.9 %SIB, $n=78$; CON-QTP-Repeated 23.8 ± 2.6 %SIB, $n=92$; $t_{(301)}=2.0$; $p=0.084$; Figure 12E-G, Table 5).

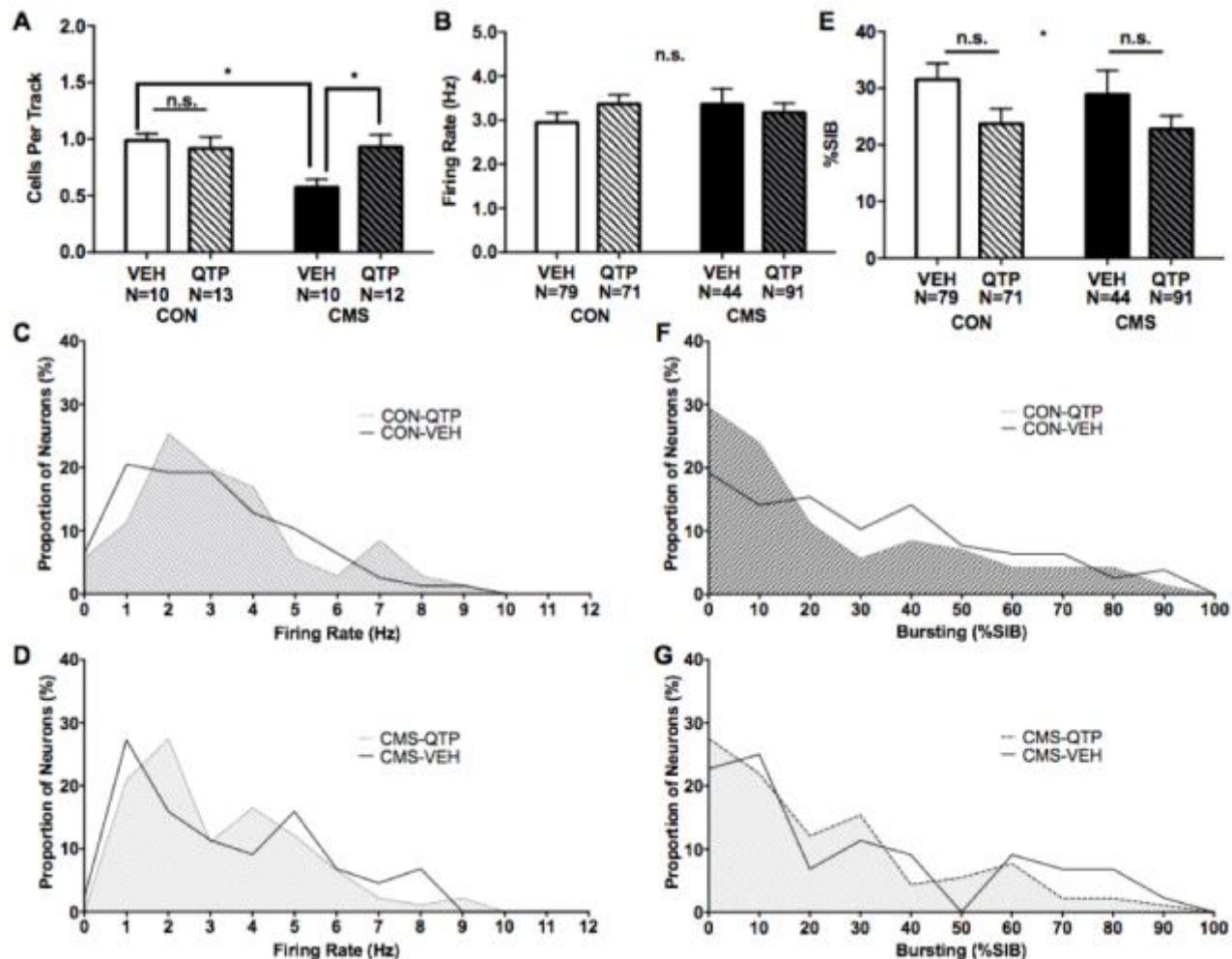


Figure 12. Impact of Repeated Quetiapine Administration in Normal and Chronically Stressed Rats

Repeated administration of quetiapine for 21+ days reversed the CMS-induced decrease in dopamine population activity without affecting this measure in controls. A) Dopamine population activity was significantly increased in CMS-exposed rats following repeated quetiapine treatment, but was unchanged in control rats treated repeatedly with quetiapine. B-D) Average dopamine neuron firing rate was unchanged by chronic stress or quetiapine. E-G) Despite a significant main effect of Drug in 2-way ANOVA, post-hoc testing did not reveal significant changes in proportion of burst activity in either control or CMS rats treated repeatedly with quetiapine as compared to their respective control group treated repeatedly with vehicle.

Table 5. Summary of Dopamine Neuron Data for Repeated Quetiapine

	<u>CON-VEH-Chronic</u>	<u>CON-QTP-Chronic</u>	<u>CMS-VEH-Chronic</u>	<u>CMS-QTP-Chronic</u>
Cells Per Track	0.99 ± 0.060	0.92 ± 0.10	0.58 ± 0.070	0.93 ± 0.11
2-Way Interaction	F _(1,42) = 5.2 ; p = 0.028 ^a	-	-	-
Drug Effect	F _(1,42) = 2.3; p = 0.13 ^a	t ₍₄₂₎ = 0.53; p = 0.84 ^b	-	t ₍₄₂₎ = 2.7; p = 0.021 ^b
Stress Effect	F _(1,42) = 4.5; p = 0.040 ^a	-	t ₍₄₂₎ = 2.9; p = 0.011 ^c	t ₍₄₂₎ = 0.11; p = 0.99 ^c
Firing Rate (Hz)	2.9 ± 0.21	3.4 ± 0.21	3.4 ± 0.34	3.2 ± 0.21
2-Way Interaction	F _(1,42) = 1.5 ; p = 0.21 ^a	-	-	-
Drug Effect	F _(1,42) = 0.22; p = 0.64 ^a	- ^d	-	- ^d
Stress Effect	F _(1,42) = 0.23; p = 0.63 ^a	-	- ^d	- ^d
Bursting (%SIB)	31.6 ± 2.9	23.8 ± 2.6	28.9 ± 4.2	22.8 ± 2.4
2-Way Interaction	F _(1,301) = 0.077 ; p = 0.78 ^a	-	-	-
Drug Effect	F _(1,301) = 5.4; p = 0.020 ^a	t ₍₃₀₁₎ = 2.0; p=0.084 ^b	-	t ₍₃₀₁₎ = 1.3; p = 0.33 ^b
Stress Effect	F _(1,301) = 0.36; p = 0.55 ^a	-	- ^d	- ^d
Group N (Rats)	N= 10 rats, 78 cells	N = 13 rats, 92 cells	N = 10 rats, 44 cells	N = 10 rats, 91 cells

^aMain effects among all groups;

^bSidak's post-hoc test for drug effect (within same stress category);

^cSidak's post-hoc test for stress effect (within same drug group);

^dMain effect not significant, post-hoc test not performed

Rats exposed to CMS that received 21+ days of vehicle injections showed reduced dopamine neuron population activity compared to control rats treated repeatedly with vehicle (CON-VEH-Repeated 0.99 ± 0.06 CPT, $n=10$; CMS-VEH-Repeated 0.58 ± 0.07 CPT, $n=10$; $t_{42}=2.9$, $p=0.011$; Figure 12A, Table 5). Consistent with the acutely-treated vehicle groups, CMS-exposed rats repeatedly treated with vehicle were similar to control rats repeatedly treated with vehicle in average dopamine neuron firing rate (CON-VEH-Repeated 2.9 ± 0.21 Hz, $n=78$; CMS-VEH-Repeated 3.4 ± 0.34 Hz, $n=44$; 2-way ANOVA $p>0.05$ for main effects of stress or interaction; Figure 12B-D, Table 5) and proportion of burst activity (CON-VEH-Repeated 31.6 ± 2.9 %SIB, $n=78$; CMS-VEH-Repeated 28.9 ± 4.2 %SIB, $n=44$; 2-way ANOVA $p>0.05$ for main effects of stress or interaction; Figure 12E-G, Table 5).

In rats exposed to CMS, repeated treatment with quetiapine increased dopamine population activity compared to CMS-exposed rats that received repeated treatment with vehicle (CMS-VEH-Repeated 0.58 ± 0.07 CPT, $n=10$; CMS-QTP-Repeated 0.93 ± 0.11 CPT, $n=13$; $t_{42}=2.7$, $p=0.021$; Figure 12A, Table 5). This increase reversed the CMS-induced attenuation of dopamine neuron population activity to levels identical to that of control rats. Compared to rats treated repeatedly with vehicle, the rats that received repeated treatment with quetiapine did not show significantly altered average dopamine neuron firing rate (CMS-VEH-Repeated 3.4 ± 0.34 Hz, $n=44$; CMS-QTP-Repeated 3.2 ± 0.21 Hz, $n=91$; 2-way ANOVA $p>0.05$ for main effects of drug or interaction; Figure 12B-D, Table 5) or proportion of burst activity (CMS-VEH-Repeated 28.9 ± 4.2 %SIB, $n=44$; CMS-QTP-Repeated 22.8 ± 2.4 %SIB, $n=91$; $t_{301}=1.3$, $p=0.33$; Figure 12E-G, Table 5), despite a significant main effect of drug treatment across all groups for bursting ($F_{(1,301)}=5.4$; $p=0.020$).

3.3.3 Experiment 3: Repeated Quetiapine Does Not Induce Depolarization Block in Normal Rats

Repeated administration of antipsychotic drugs, including high doses of second generation agents such as quetiapine, have been shown to induce depolarization block following >21 of administration to normal rats. However, the induction of depolarization block following lower doses of quetiapine such as 10 mg/kg as used at present has not been examined. Thus, for a portion of the normal rats that received repeated treatment with quetiapine in Experiment 2, we tested for the presence of depolarization block at the conclusion of the dopamine neuron recording. Following an initial recording of dopamine neurons from 6-9 tracks within the A10 region, a presynaptic dose of apomorphine (40-80 µg/kg) was administered via the lateral tail vein (infusion verified by a decrease in firing rate of a single recorded dopamine neuron), and an additional 6-9 tracks of dopamine neuron recording was performed, allowing for pre- and post-apomorphine assessments of dopamine neuron population activity within the same animal. We observed no change in dopamine neuron population activity following apomorphine administration (Pre-APO 0.75 ± 0.11 CPT; Post-APO 0.79 ± 0.14 CPT; paired $t_3=1$, $p=0.39$; Figure 14), suggesting that depolarization block did not occur following repeated administration of 10mg/kg quetiapine.

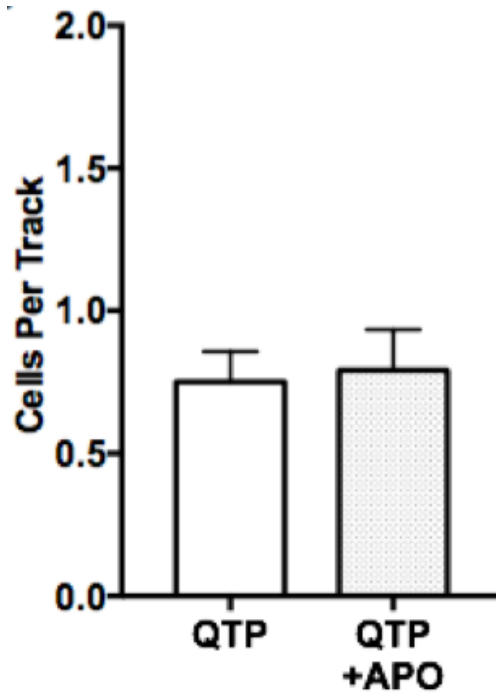


Figure 13. Depolarization Block Does Not Occur with Repeated Antidepressant Doses of Quetiapine

VTA dopamine neuron population activity was unchanged in rats sampled both pre- and post-apomorphine administration, suggesting no depolarization block was evident in control rats treated with quetiapine for 21+ days.

3.4 DISCUSSION

In this study, we found divergent effects of acute and repeated administration of quetiapine in normal and CMS-exposed rats. While acute quetiapine increased dopamine population activity in normal rats, it had no effect on CMS-exposed rats. Conversely, quetiapine administered repeatedly was uniquely capable of restoring normal levels of dopamine neuron population activity in CMS-exposed rats, but had no persisting effects on population activity in normal rats. Furthermore, we show that the normalization of dopamine neuron population activity in normal rats administered quetiapine for 21 days is unlikely to be due to the induction of depolarization block in VTA dopamine neurons.

Prior studies have administered quetiapine to CMS-exposed rats and observed behavioral effects consistent with an antidepressant action (Orsetti et al., 2007). However, as far as we are aware, this is the first study to examine the electrophysiological impact of quetiapine on the dopamine system in rats exposed to an animal model of depression. Our results suggest that quetiapine has significantly different effects on the dopamine system in CMS-exposed rats as compared to its effects in normal rats. These data add to existing literature on the dopamine system impact of acute and repeated quetiapine treatment in normal rats. These studies found that acute administration of quetiapine at doses of 10 mg/kg or greater will induce acute increases in population activity of the A10 (i.e., VTA) but not A9 (i.e., substantia nigra) dopamine neurons (Goldstein et al., 1993). Moreover, the impact of repeated administration of quetiapine was examined at increasing dosages, and found to induce depolarization inactivation only when administered at doses of 40 mg/kg or greater (Skarsfeldt, 1995). This higher dose is more in line with the doses used clinically in the treatment of psychosis or acute mania (i.e., 600-800mg), whereas the dose used in the treatment of depression (i.e., 300mg) is more in line with the 10

mg/kg dose used in the present study, as measured by D2 receptor occupancy (Kapur, VanderSpek, Brownlee, & Nobrega, 2003).

The mechanism of the increase in dopamine population activity with acute or repeated treatment with quetiapine, like other D2RA's, is thought to be primarily due to heightened presynaptic drive via feedback loops originating in the limbic striatum, as lesions of these feedback pathways block the antipsychotic drug-induced increase in population activity (Bunney & Grace, 1978). Similarly, the mechanism for the induction of depolarization block is thought to be a markedly enhanced overdrive of this pathway, resulting in elevation of the resting membrane potential of dopamine neuron cell bodies to levels that do not allow for the propagation of electrical impulses due to persistently inactivated voltage-gated sodium channels (Grace et al., 1997). Thus, one plausible therapeutic mechanism for quetiapine and other D2RA medications that function as antidepressants is that at the low doses used, they are utilizing these same feedback pathways to stimulate dopamine neuron activity, but not overdriving neurons to the point of depolarization block. Moreover, because many of these neurons are starting in a pathologically hyperpolarized state (reflecting the lower number that are spontaneously active), the effect of persistent afferent stimulation would be expected to ultimately lead to normalized levels of dopamine neuron population activity. We propose that the low level of D2 blockade without inducing depolarization block occurring with low doses of quetiapine is compensated homeostatically by increased D2 receptor number, increased dopamine synthesis, and increased release. As a result, the normalization of dopamine neuron population activity produces a greater restoration of phasic dopamine system responsivity to afferent drive (Grace, 2016) compared to the low tonic level of receptor blockade.

The principle brain region believed to impact VTA dopamine population activity is the ventral pallidum (VP), as pre-infusion of the GABA-A receptor antagonist into the VP blocks the increase in dopamine population activity observed following acute administration of sertindole or haloperidol (Valenti & Grace, 2010). Thus, D2RAs are hypothesized to enhance accumbal inhibitory drive of the VP, releasing dopamine neurons from VP inhibition and subsequently increasing the number that are spontaneously active. We have shown previously that the VP is also a critical node in the pathway driving the CMS-induced hypodopaminergic state (Chang & Grace, 2014). Therefore, these collective data lead to the intriguing possibility that convergent and counteracting effects on the VP is the mechanism of action of these D2RA medications' antidepressant effects, with CMS increasing and quetiapine decreasing VP inhibitory tone over VTA dopamine neurons.

The approach of using subsets of D2RA medications for an antidepressant effect is a promising therapeutic mechanism. The present study focused only on quetiapine rather than other D2RAs such as haloperidol or clozapine because quetiapine has been shown to possess antidepressant effects clinically (Komossa, Depping, Gaudchau, Kissling, & Leucht, 2010). Like other second-generation atypical antipsychotic drugs, quetiapine induces changes in activity relatively selectively at VTA (i.e. A10) dopamine neurons, whereas first generation antipsychotics such as haloperidol act on both the nigrostriatal (i.e., A9) and mesolimbic A10 dopamine neurons (Chiodo & Bunney, 1983; Goldstein et al., 1993), which is the likely reason for the greater preponderance of motoric side effects of the first generation antipsychotics. Moreover, the binding profile of quetiapine is much different than other D2RA antipsychotic drugs, even within the second generation (atypical) class (Lako, van den Heuvel, Knegtering, Bruggeman, & Taxis, 2013). Thus, quetiapine demonstrates a unique receptor occupancy curve wherein it binds

fleetingly with high potency and rapid dissociation, rather than a prolonged occupancy seen with other compounds (Kapur, Zipursky, Jones, Shammi, et al., 2000). It is possible that these additional collective qualities may account for the unique ability of quetiapine to act as an antidepressant medication while haloperidol and other more traditional antipsychotic drugs are not effective as antidepressants.

In conclusion, the present data support unique and divergent effects of acute and repeated administration of the D2RA quetiapine in normal and chronically stress-exposed rodents. These data offer compelling evidence for further investigating the dopaminergic component of the therapeutic mechanism of select D2RA medications, and highlight the importance of conducting these studies using an animal model of depression in addition to normal rats.

4.0 GENERAL DISCUSSION

4.1 SUMMARY OF FINDINGS

In the first portion of this dissertation, we evaluated the dopamine regulating roles of two brain regions with significant clinical evidence for involvement in MDD. We found these two regions have distinctly different effects on subpopulations of dopamine neurons. Next we expanded on these observations from normal rats to investigate whether either region drives CMS-induced decreases in dopamine population activity. We observed that inactivation of the ILPFC but not LHb reversed CMS-induced deficits in dopamine population activity, suggesting a greater role for the ILPFC relative to the LHb in CMS-induced dopamine system deficits. Finally, we examined the effects of acute and repeated treatment with quetiapine in rats exposed to CMS. We found that repeated but not acute treatment restored normal dopamine neuron population activity in CMS-exposed rats. We now discuss the implications of these findings for the circuitry of MDD and its treatment.

4.2 CIRCUITRY IMPACTING DOPAMINE IN MDD

4.2.1 ILPFC/BA25

The ILPFC has received considerable attention recently as an important node in depressive features and antidepressant-related effects in rodent studies. The unique ability of the ILPFC to

restore dopamine activity after CMS in this work helps clarify a number of recent studies. Ferenczi et al. (2015) examined the impact of ILPFC activation on dopamine-related behavioral measures of reward sensitivity relevant to depression, as well as the ability of ILPFC activation to modulate dopaminergic drive on downstream targets as assessed via opto-fMRI in one of the first applications of the approach. They found that induction of moderate but sustained elevation of ILPFC activity using a step-function opsin induced behavioral deficits in sucrose preference, social interaction, and real-time conditioned place preference for optically driven dopamine stimulation, suggesting that ILPFC activation dampened the typical dopaminergic response associated with these activities. This interpretation was further supported by opto-fMRI data suggesting a diminished post-synaptic induction of activity in the striatum. Finally, local field potentials recorded in the ILPFC and striatum showed an increase in gamma band coherence between these regions during ILPFC activation, suggestive of enhanced functional connectivity. Thus, these data support a functional significance for our observed decrease in dopamine population activity following ILPFC activation at both the behavioral and systems levels.

It is also interesting to compare the results from our studies to recent papers investigating antidepressant mechanisms of ILPFC DBS or ketamine microinfusion. Hamani et al (2010) showed that deep brain stimulation but not inactivation of the entire mPFC was able to reverse CMS induced behaviors (Hamani et al., 2010). However, we have previously shown that inactivation of the two regions comprising the mPFC - i.e. the ILPFC and the prelimbic prefrontal cortex (PLPFC) - has opposite effects on the dopamine system, with ILPFC inhibition increasing and PLPFC inhibition decreasing VTA dopamine neuron population activity (Patton et al., 2013). Thus that group's concurrent inactivation of the PLPFC likely canceled out any antidepressant effect of ILPFC inactivation. More recently, Fuchikami et al (2015) showed that microinfusion of

ketamine into the ILPFC induced antidepressant effects comparable to systemic administration, and that 1-hour of high frequency ILPFC stimulation using optogenetics also mimicked the antidepressant effect of ketamine examined multiple days later. However, the stimulation protocol used in this study aimed to recreate ketamine's effect on plasticity within ILPFC microcircuitry (Fuchikami et al., 2015), rather than simple activation of the region as was performed by Ferenczi et al (2015) and is more reflective of the NMDA microinfusion used in the present work. Thus it is not surprising that they observed an anti-depressive effect with their ILPFC stimulation protocol. Finally, that study is further complicated by the recent discovery that the antidepressant effect of ketamine is not due to its NMDA antagonism properties per se, but rather the effects of a metabolite (Zanos et al., 2016).

4.2.2 LHB

Although the LHB has been an intense focus of investigation, the present study is the first to systematically evaluate the changes in firing properties of the overall population of VTA dopamine neurons that occur with persistently elevated LHB activation over a period of 1-3 hours. In contrast, prior studies examining the ability of the LHB to inhibit dopamine neurons have focused on reductions in firing rate with LHB stimulation. The most likely mechanism for this inhibition would be the LHB activating the RMTg, leading to enhanced efferent inhibitory drive over VTA dopamine neurons. Based on our observed data, we propose this effect would be strongest when directed at dopamine neurons located in lateral VTA.

Based largely on data collected in non-human primates, a role has been proposed for the LHB for inhibiting dopamine neurons in response to a negative reward prediction error, i.e. to signal disappointment when an expected reward was not delivered. Thus, considering that the

behavioral roles of the LHb that have been proposed emphasize phasic responses to inhibit dopamine neurons acutely in response to individual stimuli, rather than for creating a set point at steady-state, one might think it seems counterintuitive that LHb activation induced a reduction in the number of dopamine neurons firing but did not reduce their firing rate or amount of bursting. In contrast, a slight increase in firing rate in medial dopamine neurons was observed.

In order to reconcile these findings, one must consider the nature of the circuit interrogation performed in the current experiments. In this study, we chose to use NMDA to create a more persistent increase in LHb activity, rather than phasic increases in activity as have been performed previously using optogenetics or electrical stimulation. A manipulation such as we performed would therefore be expected to provide a better estimation of the persisting effect of LHb activity as would be expected to occur if this region were persistently overactive, as has been hypothesized to be the case in MDD. Thus, the findings from this experiment suggest that the LHb, when tonically activated at higher levels, regulates tonic drive of dopamine neurons located only in lateral VTA. As discussed earlier, these neurons have a greater extent of projections to associative striatum and involvement in cognitive tasks, whereas the more medially located dopamine neurons project more heavily to ventral striatum and are more likely to invigorate motivational states. Thus, the present findings suggest pathological activation of the LHb would be more likely to result in diminished cognitive reserve than motivational drive, per se. Furthermore, the lack of overlap of the neurons inhibited by LHb activation and those inhibited by CMS suggest the LHb would not be involved in mediating chronic stress-induced dopamine system hypofunction. This hypothesis was supported by our data demonstrating that LHb inactivation did not restore dopamine neuron population activity to normal levels in rats exposed to CMS.

Importantly, our results do not rule out a role for the LHb in all aspects of depression. Rather, we have shown that the LHb is not involved in lowering the gain of the dopamine system in response to chronic stress. This actually makes sense given that most literature ties the LHb to decision making and other cognitive functions, and not modulating motivational drive. Finally, there is still potential for the LHb to be involved in the pathophysiology of depression in other ways such as mediating dysfunction in the serotonin system via its influence over the dorsal raphe.

4.2.3 ILPFC-LHb Interactions

Given the existence of a projection from the ILPFC to the LHb that drives FST immobility (Warden et al., 2012), an intriguing consideration could be that this projection has some role in driving dopamine neuron inhibition. However, our present data would argue against this notion on two grounds. First, if the ILPFC to LHb projection were driving a decrease in dopamine population activity, one would expect to see overlap in the location of the dopamine neurons inhibited when either the ILPFC or LHb were activated in normal rats. However, in the present data, ILPFC activation inhibited dopamine neuron population activity in medial VTA and increased bursting in lateral VTA, whereas LHb activation inhibited dopamine neuron population activity exclusively in lateral VTA and slightly increased the firing rate of dopamine neurons in medial VTA. Thus there were no common effects on VTA dopamine neurons with activation of these two structures to suggest a shared projection pathway. Second, if the ILPFC to LHb projection were driving the observed decrease in dopamine neuron population activity in CMS-exposed rats, one would expect to see restoration of normal dopamine neuron population activity by interrupting this circuit with inactivation of either the LHb or ILPFC. However, we found that in rats exposed to CMS, inactivation of only the ILPFC restored dopamine neuron population activity to normal levels,

whereas LHb inactivation had no effect. Thus, while the ILPFC to LHb projection may possess the ability to drive depressive-related behaviors, the present results do not support a role for this circuit in altering dopamine neuron firing properties.

4.2.4 Integration of Circuitry Regulating Dopamine in CMS

Our data on the effects of inactivating the ILPFC or LHb in rats exposed to CMS adds to a growing body of literature on the circuitry regulating dopamine population activity and potential points of pathology in disease states that alter dopamine system function (Grace, 2016).

Specifically, the finding that ILPFC inactivation in CMS-exposed rats restores dopamine population activity by potentially increasing the activation of dopamine neurons across the extent of the VTA is particularly intriguing. It has been demonstrated in experiments with normal rats that inactivation of the ILPFC drives an increase in the number of spontaneously active dopamine neurons via a circuit that traverses the thalamic nucleus reunions en route to the vSub of the hippocampus (Zimmerman & Grace, 2016). Given that the vSub has the ability to drive increases in dopamine population activity across the medial/lateral extent of the VTA (Valenti et al., 2012), the present data argue for a role of the vSub in restoring normal levels of dopamine population activity after CMS via ILPFC inactivation.

In other neuropsychiatric disorders, it has become apparent that most treatments do not reverse the site of primary pathology, but rather induce offsetting synaptic plasticity (Bowers & Ressler, 2015). Thus, treatments that reduce BA25 activity may result in a resolution of depressive symptoms, however, the underlying susceptibility of that individual to develop depression still remains (Holtzheimer & Mayberg, 2011b). This would be expected to contribute to the high rate

of recurrence of depression in patients who previously underwent successful treatment, particularly when they are not maintained on treatment following the resolution of symptoms.

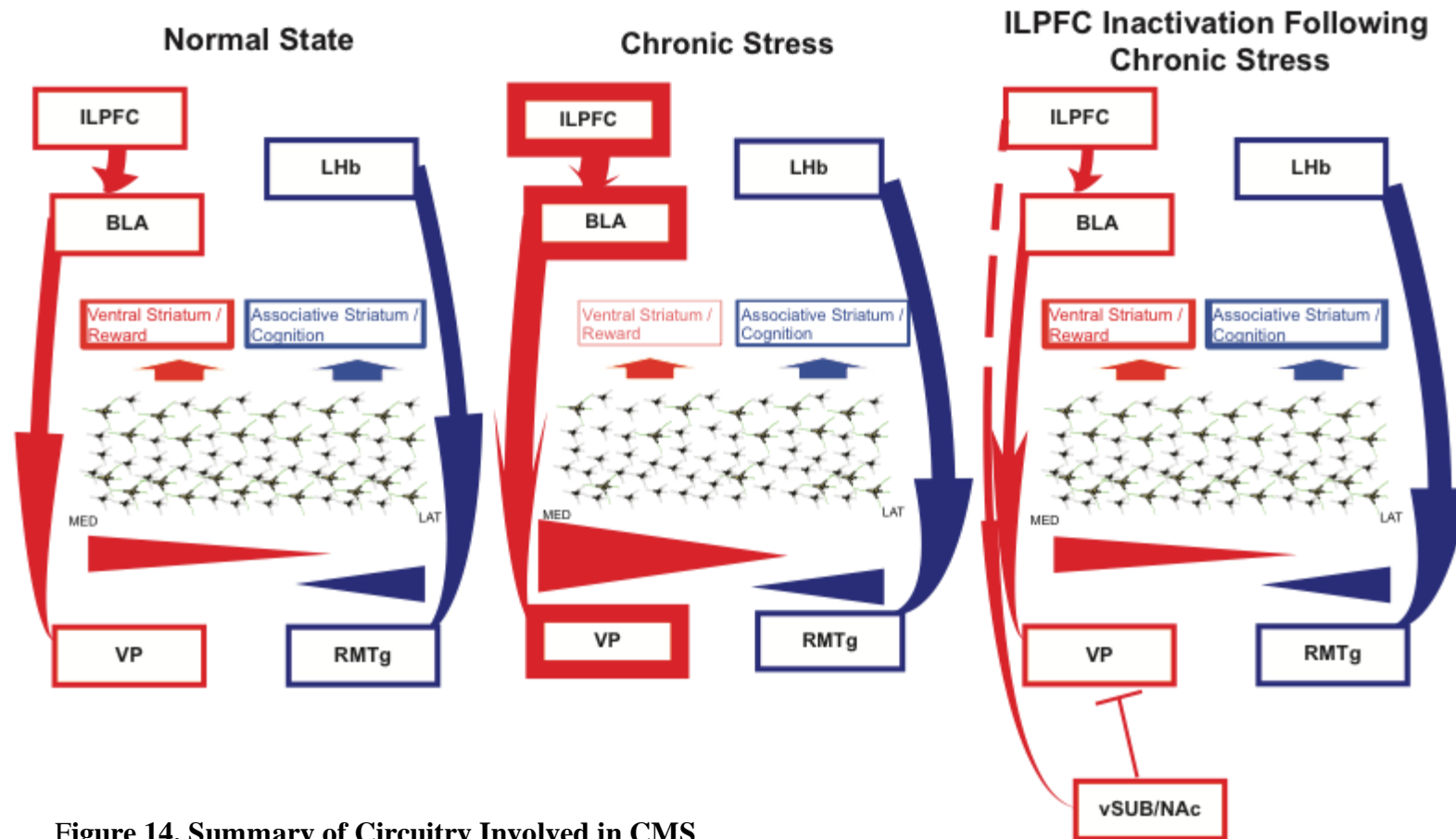


Figure 14. Summary of Circuitry Involved in CMS

In the normal state, afferent regions are functioning at baseline levels, resulting in normal levels of VTA dopamine neuron population activity. When activated, the ILPFC preferentially inhibits dopamine neurons located in medial VTA, whereas the LHb preferentially inhibits dopamine neurons located in lateral VTA. Chronic stress leads to overdrive of the ILPFC, which via the BLA and VP, reduces dopamine population activity in medial VTA. The functional implication of the transition of these dopamine neurons into a silent inactive state is decreased availability to fire when presented with reward-related stimuli. This is proposed to correlate with the blunted response to rewarding stimuli reported by patients with depression. ILPFC inactivation after CMS halts this pathology as well as activates the countervailing vSub/NAc pathway to restore normal VTA activity.

4.3 QUETIAPINE CIRCUIT EFFECTS IN MDD

4.3.1 Insights from Mechanisms in Schizophrenia

The antidepressant effect of quetiapine was discovered incidentally in studies of its use for patients with schizophrenia. While the dopamine system pathology in schizophrenia and MDD are most likely different, if not opposite, we can still gain insight into the mechanism of quetiapine's antidepressant effect by examining its impact on dopamine regulating circuitry in the context of schizophrenia.

4.3.1.1 Time Course

In schizophrenia, there is increased dopamine system activity, which animal models suggest is driven by an increase in the number of dopamine neurons that are spontaneously active (Grace, 2015). One currently accepted conceptualization of how this manifests symptomatically as schizophrenia is that the excessive dopamine signaling results in the attribution of “aberrant salience” to environmental stimuli that otherwise should not be of significant interest to the subject, which is expected to result in positive symptoms such as paranoia (Kapur, 2003). D2RAs reduce dopamine transmission, with resulting decreases in positive symptoms (Kapur, Agid, Mizrahi, & Li, 2006).

The mechanism of quetiapine's antipsychotic effect is thought to be due to blockade of dopaminergic transmission in the striatum, which leads to enhanced feedback to further increase dopaminergic drive, and ultimately induces a state of depolarization block (Grace et al., 1997).

Clinically this effect is observed within hours to days (Kapur et al., 2005). This was perplexing, because when the D2RA induction of depolarization block is examined in normal rats, this phenomenon takes 21 days to develop (Bunney & Grace, 1978). The notion that the time course of D2RA effects may differ between normal subjects and those with schizophrenia emerged when the effects of D2RA medications were examined in a rodent model of schizophrenia that features a prominent hyperdopaminergic state. Using the MAM model, it was observed that D2RAs induced depolarization block in dopamine neurons after the first dose, whereas in normal rats, this process takes 21 days (Valenti, Cifelli, et al., 2011). Thus, because the set point of the dopamine system was already at an elevated state, it appears the system was more easily pushed into depolarization block.

4.3.1.2 D2R Occupancy

One of the most interesting features of quetiapine as compared to other medications in the D2RA class is the time course of its binding to the dopamine D2R. Thus, for most D2RA APDs, binding is initiated and persists at a relatively stable level, with gradual drop off as the drug is metabolized (Ginovart & Kapur, 2012). Conversely, quetiapine binds to the D2R at a high level of occupancy within the first 4 hours, and then its occupancy rapidly decreases (Kapur, Zipursky, Jones, Shammi, et al., 2000). These data were intriguing, because it was thought that in order for a D2RA to have an APD effect, it needed to have a persistent D2R occupancy above 65% (Kapur, Zipursky, Jones, Remington, & Houle, 2000). However, quetiapine appeared to only maintain occupancy above 60% for a very short period of time. This observation led to the proposal that persistently high levels of D2R occupancy were not required for antipsychotic effects to be maintained over time (Remington & Kapur, 2010). Instead, it seems that induction of therapeutic depolarization

block of dopamine neurons can be achieved reliably with only brief but repeated exposure to high levels of D2R occupancy (Tauscher-Wisniewski et al., 2002).

4.3.2 Synthesis of Potential Antidepressant Mechanism

As summarized above, most of our understanding of the pharmacological properties of quetiapine were revealed from studies of the mechanisms of its antipsychotic effect, and these efforts yielded a number of observations that should prove useful in discerning its antidepressant effect.

4.3.2.1 Impact of State of Dopamine System

As discussed previously, the baseline state of the dopamine system at time of D2RA administration has a profound impact on the subsequent effect on dopamine neuron population activity. While in schizophrenia, the dopamine system is *hyperactive*, in MDD, the dopamine system is *hypoactive*. Our lab's results from converging lines of research across different stress-induced animal models of MDD suggests that this dopamine system hypoactivity is observed clinically because fewer dopamine neurons are spontaneously active in patients with MDD, resulting in lower levels of tonic activation of the dopamine system as well as reduced capacity for phasic response, given that only spontaneously active dopamine neurons are capable of burst firing to salient stimuli.

In the present experiments, we observed that quetiapine administered at antidepressant doses (10 mg/kg) to CMS-exposed rats had no acute effect on the number of spontaneously active dopamine neurons, but restored a normal number of spontaneously active dopamine neurons with repeated administration for 21+ days. These data suggest that in a hypodopaminergic state, the time course for dopamine system recovery with D2RA treatment is longer than in a hyperdopaminergic state, where effects are observed immediately.

It is tempting to speculate that perhaps the substantially lower dose of quetiapine when used for antidepressant treatment may be to blame for the lack of an acute effect. However, the collective data from a multitude of clinical trials would argue against this. Thus, in the patients studied in the pivotal placebo-controlled randomized clinical trials, doses of 150 mg/day were most effective when quetiapine was used as monotherapy, and doses of 300 mg/day were most effective when quetiapine was used as augmentation to existing SSRI or SNRI therapy. These doses are far lower than those used for quetiapine in the treatment of schizophrenia (i.e. >800 mg/day), and increasing doses resulted in no additional antidepressant effect. Thus, it would appear that the lack of an acute increase in dopamine population activity in CMS-exposed rats is more likely due to a greater time requirement for duration of treatment, rather than an increased dose requirement per se. This notion is also supported by the clinical trials, where antidepressant effects were observed over 1-6 weeks, with very few patients reporting acute improvements in mood. The reason for this longer time course remains unclear, but may have to do with time requirements for plasticity to occur in striatal feedback circuitry, discussed in greater detail below.

The reason for the difference in dose requirements in the clinical trials when quetiapine was used as augmentation vs. monotherapy remain unclear. One possibility could be that there is greater degree of dopamine system pathology in the patients who failed initial SSRI/SNRI therapy and thus qualified for a trial of quetiapine augmentation, and this was better addressed by a higher dose of quetiapine. Another possibility could be that concurrent treatment with other antidepressant medications interfered with quetiapine's effect on a pharmacological basis, as increased synaptic levels of serotonin due to SSRI therapy could potentiate inhibitory effects of serotonin on dopamine release, thus necessitating a larger quetiapine dose to overcome this added dopamine-reducing effect.

4.3.2.2 Common Circuitry of Dopamine Regulation

All D2RAs induce increases in dopamine population activity via induction of greater NAc inhibitory tone over the VP and subsequent disinhibition of VTA dopamine neurons, which appears to result from striatal post-synaptic D2R blockade. We have shown that in rats exposed to CMS, there is increased VP inhibition of VTA dopamine neurons, which is the reason that fewer are spontaneously active (Chang & Grace, 2014). These effects occurring concurrently raise the potential for an intriguing interaction between these two opposing influences over VP activity, wherein CMS drives and quetiapine inhibits VP activity. Such an effect would explain why quetiapine worked to increase dopamine population activity in CMS-exposed rats, i.e. that it resulted in strengthening of opposing synaptic plasticity of NAc afferents onto the VP, thus restoring normal levels of VP inhibitory tone onto the VTA. Furthermore, this hypothesis is supported by both clinical and pre-clinical data supporting the effectiveness of quetiapine in presently depressed/CMS-exposed subjects, as well as in maintenance therapy of patients who remitted from depression or rats treated at the onset of exposure to CMS (Orsetti et al., 2007). Thus, it would appear that quetiapine has the ability both to counteract existing pathologically increased VP activity, as well as prevent its onset in individuals susceptible to its development.

4.4 FUTURE DIRECTIONS

4.4.1 Circuit Explorations

A major point that has emerged from our lab's inquiry into the circuitry driving downregulation of the dopamine system in depression is how specifically information flows through the different nodes that we have identified. Thus, while the impact of ILPFC activation has been shown to depend on intact glutamatergic afferents to the BLA, and the effect of BLA activation depends on glutamatergic afferents to the BLA, it remains possible that other intermediary regions could also be involved. In one study, the projection from ILPFC to amygdala was shown to reduce anxiety, but overlap between specific amygdala subregions our group has examined and those studied in that paper remain unclear (Adhikari et al., 2015). This is important, as different circuits through a given region often have been shown to have distinct or opposite effects (Stuber et al., 2011).

4.4.2 Pharmacology Explorations

While the present study offers some insight into the potential mechanism of quetiapine's antidepressant effect, many questions still remain. Among the most critical is a determination of what specific pharmacological properties of quetiapine drive its uniquely potent antidepressant effect among the D2RA medications.

4.5 FINAL REMARKS

In this dissertation research we performed an in-depth examination of the impact of clinically relevant circuits modulating dopamine function and their relation to the CMS rodent model of depression. We also assessed the effects of novel D2RA treatment approaches for MDD and possible mechanisms of their effect in the CMS model. Together, these data offer insights into stress-induced dopamine system deficits in the context of MDD. Future efforts will continue to leverage these pathways to explore mechanisms of dopaminergic changes in MDD and guide efforts towards their remediation.

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